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ION ANTAGONISM AND THE FROG HEART

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The current concept of ion antagonism seems to be somewhat similar to that held by Sydney Ringer, namely, that a given pair of ions oppose each other in their action on all activities and properties of the heart. While it is probably realized that this is not strictly true, there is little experimental information to indicate the extent of this opposing action.

The present report is an attempt to describe the concomitant action of one pair of ions, Ca and K, on the frog heart. Two[1] possible manifestations of antagonism are considered: 1, toxicologic, and 2, physiologic.

The experimental part of the present communication concerns only toxicologic effects. Specifically, the attempt is here made to determine if the physiologic limits of Ca^{++} concentration can be modified by changing the K^{+} concentration.

EXPERIMENTS. All experiments were carried out on the frog (*Rana pipiens*) heart. The heart was perfused through the sinus venosus in the manner previously used (Spealman, 1938).

The observations made were to determine if a given solution was "toxic". The primary criterion was the heart rate, i.e., the rate of beat of the sinus venosus; for it has been shown (Daly and Clark, 1921; Spealman, 1938) that excessively high or low concentrations of any one kind of ion in an otherwise normal Ringer's solution causes slowing or complete arrest of the heart.

The procedure was to perfuse the heart with the chosen experimental solution after observations of the rate of the heart perfused with the control Ringer's solution had been made. The usual length of time of perfusion was 30 minutes, with observations on the heart recorded every 10 minutes. If the heart rate showed no change, another experimental perfusion fluid was tried; however, if the heart showed an appreciable decrease in rate, it was immediately perfused with the control Ringer's solution to determine if the change had been due to the experimental solution or to some other factor. Often it was not necessary to perfuse an experimental solution as long as 30 minutes to demonstrate that it was toxic; but occasionally it was necessary to perfuse the heart longer in order to

[1] Ringer (1887) divided the possible antagonistic action into these two sub-divisions.

make a decision. Observations on the presence and degree of heart block were also made.

Two series of perfusion fluids were used. In each series the concentration of $NaHCO_3$ and of NaCl were the same, 2 mM/L and 110 mM/L respectively. In one series the KCl concentration was 1 mM/L and in the other, it was 4 mM/L. The $CaCl_2$ concentration was varied in each series from 0.125 to 8.00 mM/L. For each series, the solution containing 2.00 mM/L of $CaCl_2$ was used as the control solution.

Thus two sets of experimental solutions were employed, one having a K^+ concentration near the upper limit of normal and one having a K^+ concentration near the lower limit of normal. For each of these two sets of solutions, the limits

TABLE 1

Heart rate. The heart rate in the control Ringer's solution (2.0 mM/L Ca ion) in each case is taken as 1.00. The subscripts indicate the experimenter's estimate of the significance of the decrease in rate, when such factors as spontaneous variation in heart rate, progressive nature of the change, etc. are considered. (d) represents a significant decrease, (n) no significant decrease, — indicates that no reading was taken.

Heart	0.125	0.25	0.50	2.00	4.00	8.00
			Calcium ion concentration in mM/L			
			a. K ion concentration of all solutions, 1 mM/L			
1	0.62_d	1.02_n	—	1.00_n	1.00_n	0.72_d
2	0.73_d	0.94_n	1.00_n	1.00_n	0.86_n	0.60_d
3	0.44_d	0.89_n	—	1.00_n	0.91_n	0.69_d
4	0.95_n	1.01_n	0.92_n	1.00_n	—	0.63_d
5	0.48_d	0.81_n	—	1.00_n	0.93_n	0.38_d
			b. K ion concentration of all solutions, 4 mM/L			
1	0.55_d	0.96_n	0.93_n	1.00_n	0.84_n	0.65_d
2	0.48_d	0.83_n	0.75_n	1.00_n	0.86_n	0.65_d
3	0.78_d	0.85_n	0.67_n	1.00_n	0.95_n	0.68_d
4	0.49_d	0.93_n	0.97_n	1.00_n	0.93_n	0.54_d
5	—	0.59_d	1.13_n	1.00_n	1.14_n	0.77_n

of Ca^{++} concentration which would allow the heart to continue beating at its normal rate for a reasonable period of time was determined, as explained above.

Results. Table 1 gives the data on the behavior of the heart rate in these solutions. The data show that the limits of Ca^{++} concentration in which the heart rate is not significantly decreased from the control are apparently the same whether the solutions contained 1.00 or 4.00 mM/L of K^+.

Table 2 summarizes the data on the behavior of the heart as regards atrio-ventricular conduction. The data were obtained on the same hearts as the data of table 1. N indicates a normally behaving heart, and B indicates that block occurred. The plus signs after the B's indicate the extent of block (see legend of table). The data indicate that the region of Ca^{++} concentration in which heart

block is absent shifts to higher Ca^{++} concentrations, as the K^+ concentration of the solution is increased from 1 to 4 mM/L.

Discussion. Sydney Ringer's evidence for a physiologic antagonism between the ions was much stronger than his evidence for a toxicologic one. However, the physiologic antagonism is not so complete as is generally believed. For example, there is no physiologic antagonism between Ca^{++} and K^+ so far as the heart rate is concerned, for neither ion in physiologic concentrations affects the heart rate (Spealman, 1938). Likewise, there appears to be only a very feeble antagonism (Clark, 1926) or none at all between ions so far as the amplitude of contraction is concerned; for, although Ca^{++} influences this activity, K^+, within

TABLE 2

Heart block. N indicates heart block did not occur. B+ indicates an atrio-ventricular block of less than 2 to 1; B++, greater than 2 to 1, but not complete; and B+++ indicates a complete atrio-ventricular block. The B's with plus signs, following some of the N's in the control (2.00 mM/L Ca ion) column, indicate that block was present with this solution during the latter part of the experiment. The asterisk above and to the left of the B indicates the heart block occurred after it appeared in the control solution. — indicates no reading was taken.

Heart	0.125	0.25	0.50	2.00	4.00	8.00
			Calcium ion concentration in mM/L			
			a. K ion concentration of all solutions, 1 mM/L			
1	N	N	—	N	B++	B+++
2	B+++	B+	N	N	B+	B+++
3	N	N	—	N	B++	B+++
4	B++	B++	N	N(B+++)	—	B+++
5	B+	N	—	N(B++)	B+++	*B+++
			b. K ion concentration of all solutions, 4 mM/L			
1	B+	B++	N	N	N	N
2	B+++	B++	B+	N	N	N
3	B+++	B++	*B++	N(B++)	N	*B++
4	N	N	N	N	N	N
5	—	B++	B+	N	N	N

the physiologic range, has little (Clark, 1926) or no (Spealman, 1940) effect on the amplitude. However, certain properties or activities of the heart are apparently influenced in opposite directions by these ions. For example, Colle (1927) has shown in careful quantitative studies that Ca^{++} and K^+ change the excitability of the frog heart in opposite directions over a wide range of concentration of these ions. Likewise, the duration of the mechanical response appears to be affected in opposite senses by Ca^{++} and K^+ (Ringer, 1887). There are probably other activities and properties of the frog heart which are affected in opposite manners by these ions. It is likely that Ca^{++} and K^+ are mutually antagonistic in these cases. However, it should be emphasized that these two ions are not

mutually antagonistic within physiologic limits of concentration on all activities or properties of the heart.

The antagonism existing between toxic concentrations of these ions shows a similar situation. So far as the rhythmic function of the pacemaker of the heart is concerned (table 1), there appears to be no antagonism between Ca^{++} and K^{+}; for the limits of Ca^{++} concentration in which the heart rate is not depressed are uninfluenced by the K^{+} concentration. The results obtained in the studies of heart block do show that antagonism exists between these two ions in this case; for the range of Ca concentration in which block is absent shifts to higher values with increase in the K^{+} concentration of the solution.

SUMMARY AND CONCLUSIONS

1. The region of Ca^{++} concentration in which the pacemaker of the heart is able to maintain its rhythmicity unimpaired does not appear to be influenced by the K^{+} concentration.

2. The region of Ca^{++} concentration in which the heart is free from atrioventricular block is shifted to higher concentrations as the K^{+} concentration is increased.

3. From the discussion, it is apparent that the antagonism between Ca^{++} and K^{+} is of a limited nature in the case of the frog heart.

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A DEFECT IN THE COAGULATION MECHANISM OF SWINE BLOOD[1]

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About 3 years ago it was discovered that a considerable number of swine in the Missouri Agricultural Experiment Station herd were subject to severe and protracted hemorrhage. The abnormal individuals would sometimes bleed to death from a very slight injury, or no injury at all that could be detected. The bleeders are progeny of an inbreeding investigation and the affected animals are closely related. Hogan et al. (1) reported some preliminary diagnostic observations and concluded that the disease is similar to hemophilia.

The more important characteristics observed up to date may be summarized as follows: 1. The hemorrhagic tendency is inherited; both sexes have the coagulation defect and both transmit it. 2. The bleeding time by Duke's (2) method is normal. If the wound is more extensive, however, the bleeding time is prolonged. 3. The whole blood coagulation time is greatly extended. 4. The clot retracts normally when once formed. 5. There is no deficiency of calcium. 6. Howell's "prothrombin time" (3) is greatly prolonged. 7. Prothrombin is present in normal concentration. 8. The platelets are normal in number but they are abnormally stable. 9. Fibrinogen is present in normal amount. 10. When the blood comes in contact with injured tissues it coagulates in normal time. The blood is deficient in readily available thromboplastin.

The purpose of this paper is to describe: 1, a diagnostic test that is suitable for our purpose; 2, additional features of the bleeding abnormality.

EXPERIMENTAL. The routine of swine management, such as marking, putting rings in the nose, and castrating the males, requires the infliction of minor wounds. In addition the habits of the animals expose them to injuries that are ordinarily of no consequence but are extremely hazardous to individuals with the hemorrhagic tendency. The animals most useful for study are the ones most likely to be lost and it was necessary to devise diagnostic tests that would make it possible to protect them.

Diagnostic tests. Our early experience with prolonged hemorrhage from apparently insignificant scratches led us to believe that a bleeding time test would be satisfactory. In the spring of 1939, therefore, all of the pigs in the bleeder line were tested by cutting nicks in the edge of the ears. It was supposed that the animals which gave no indication of hemorrhage were normal but later when the boars were castrated eight that had given a normal test bled to death. Out

[1] Most of the data in this manuscript were taken from a thesis submitted by Mr. Muhrer in partial fulfilment of the requirements for the degree, Master of Arts.

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of the 23 animals which proved by hemorrhage at one time or another to be bleeders, only five were abnormal by the bleeding time test. Two of these bled to death within an hour after the nicks were made and the other three had narrow escapes but were saved by putting hemostatic clamps on each side of the wound. It is evident then that a test for the disease based upon bleeding time must be made on a relatively large wound if it is to be reliable, but a test of this kind that is reliable is too dangerous to use. The methods that have been seriously considered are described below.

1. *Needle test.* A 19 gauge petrolatum coated needle is inserted in the marginal ear vein. If the blood stops dripping from the external end of the needle because of clot formation in the needle in less than 5 minutes after the appearance of the first drop of blood, the animal is normal. If a clot does not form the animal is a bleeder.

2. *Microscopic test.* Twenty-five minutes after the blood is drawn into an iced paraffined tube, a smear is made on a glass slide, dried, stained by Wright's method (4) and examined under oil immersion. If the platelets are clumped and ragged the animal is normal. If the platelets have sharp outlines and are only grouped the animal is a bleeder.

3. *Whole-blood coagulation time.* The procedure is essentially the same as the Lee-White (5) modification of Howell's method. Five milliliters of blood are collected by venous puncture in cold paraffin coated tubes and placed in an ice bath. It has been found that the coagulation time is less than 40 minutes in a normal animal. If the coagulation time is 50 minutes or more the animal is probably a bleeder.

4. *Fibrin precipitation test.* (6) Four milliliters of blood are drawn by careful venous puncture into a 15 ml. paraffin coated centrifuge tube containing 1 ml. of 3.8 per cent sodium citrate. The tube is stoppered, the contents thoroughly mixed, cooled in a refrigerator at 5°C. for one hour and centrifuged at 2800 r.p.m. for 25 minutes in a centrifuge having a radius of 20 cm. The time of standing and the rate of centrifuging are important. One milliliter of the citrated plasma is mixed with a glass stirring rod in a 50 ml. graduated cylinder with 48 ml. of 0.9 per cent sodium chloride and 1 ml. of 2.5 per cent calcium chloride. If the plasma is normal it will form a mass of fibrin throughout causing the mixture to become turbid in about 16 minutes. The fibrin precipitation process is always complete, when normal plasma is used, in 20 minutes. On rotating the glass rod the fibrin is wrapped around it and the contents of the cylinder are clear again. It requires about 35 minutes for the precipitation of bleeder fibrin under these conditions.

In an effort to determine whether or not the proposed tests would consistently distinguish between normals and bleeders, three normals and three confirmed bleeders were tested by all four methods as shown in table 1.

Table 1 indicates that tests 1 and 4 are the most consistent, presumably because there is less danger of interfering contamination. These two tests were then applied to 74 animals, and 27 were bleeders and 39 were normal by both. In 8 cases, however, the blood continued to flow through the needle but when

tested for fibrin precipitation time the fibrin came down in less than 20 minutes. It developed later on that none of these animals was a bleeder.

Several of the animals that were diagnosed as bleeders had never shown any signs of hemorrhage, but with one exception they all developed external bleeding tendencies later. Boar 28 was the exception but when retested his blood flowed freely and the fibrin precipitation-time was thirty-five minutes. In our experience the fibrin precipitation test is more dependable than any other yet tried. Other advantages are: 1, the use of citrate rather than oxalate for the

TABLE 1

Tests for abnormal swine

NO.	ANIMAL	TEST 1 FLOW THROUGH NEEDLE	TEST 2 WHOLE-BLOOD COAGULATION TIME	TEST 3 MICROSCOPIC EXAMINATION OF PLATELETS	TEST 4 FIBRIN PRECIPITATION	REMARKS
		min.	*min.*		*min.*	
1	Normal	3	41	Much grouping	15	All reliable
2	Normal	1	13	Small groups	18	All reliable
3	Normal	2	57	Small groups	18	Test 2, failed
4	Defective	More than 5	98	Much grouping	Over 20	Test 3, failed
5	Defective	More than 5	85	Platelets single	Over 20	All reliable
6	Defective	More than 5	80	Platelets single	Over 20	All reliable

TABLE 2

Reliability of the fibrin precipitation test

ANIMAL NO.	FIBRIN PRECIPITATION TIME	DIAGNOSIS	REMARKS
	min.		
7	11	Normal	From a normal line
8	50	Bleeder	Died from hemorrhage
9	16	Normal	Did not bleed excessively when castrated
10	30	Bleeder	Bled profusely when castrated
11	8.5	Normal	From a normal line
12	48.0	Bleeder	Died from spontaneous internal hemorrhage one week after testing
13	18	Normal	From a normal line
14	36	Bleeder	Had been bleeding from gums for two days
15	16	Normal	From a normal line
16	41	Bleeder	Profuse hemorrhage from an ear injury

anticoagulant leaves a clear solution, in place of a white cloudy suspension, when the plasma is recalcified; 2, the end point is visible, very definite, and can be read without inverting the tube. This increases the accuracy because agitation accelerates the coagulation process; 3, a number of samples can be determined simultaneously; 4, the final readings do not need to be made immediately after securing the sample. After the blood is drawn it can be removed to a more convenient laboratory for completion.

Typical results are shown in table 2.

Characteristics of the bleeding abnormality. It seemed that the most useful guide in planning future work would be the experience of workers who had studied a similar abnormality, and our observations have been compared with those made on various blood-coagulation defects. Up to the present time the

TABLE 3

Quick's methods for the diagnosis of hemophilia

TESTS DESCRIBED BY QUICK	QUICK'S VALUE FOR NORMAL MAN	AVERAGE VALUE FOR 5 NORMAL SWINE	QUICK'S VALUE FOR HEMOPHILIACS	AVERAGE VALUE FOR 5 HEMOPHILIA-LIKE SWINE
Coagulation time of low speed plasma	90–125 sec.	111 sec.	165–540 sec.	255 sec.
Coagulation time of high speed plasma	105–145 sec.	171 sec.	330–900 sec.	429 sec.
Coagulation time of plasma after standing 6 hours	Small reduction from above value	126 sec.	Approaches normal	141 sec.
Whole blood coagulation time	Less than 8 min.	6.2 min.	More than 8 min.	14.0 min.
Quick's prothrombin time	11.5 sec.	9.1 sec.	12 sec.	9.2 sec.
Duke's bleeding time	Less than 4 min.	2.9 min.	Less than 4 min.	2.7 min.
Start of clot retraction	Less than 60 min.	68 min.	Less than 60 min.	58 min.

TABLE 4

Therapeutic value of blood globulin fraction

ANIMAL NO.		AMOUNT INJECTED PER LB. LIVE WT.	FIBRIN PRECIPITATION TIME		REMARKS
			Before injection	1 hr. after injection	
		mgm.	*min.*	*min.*	
17	To prevent hemorrhage after castration	5	48	28	Hemorrhage not excessive
16	To establish potency of globulin	10	41	23.5	Preparation active
8	To arrest ear hemorrhage	5	50		Hemorrhage stopped in one hour
1	To prevent hemorrhage after castration	10	28	19	Hemorrhage not excessive
18	To establish potency of globulin	10	37.5	29	Preparation active
14	To arrest a mouth hemorrhage that had been bleeding 48 hours	10	35	22.5	Hemorrhage stopped in two hours

abnormality as we have observed it does not differ in any significant way from human hemophilia, except in swine the defect is not sex-linked.

In forming an opinion as to the nature of the abnormality a few additional observations may have some significance. When there is no visible injury to explain the hemorrhage the most common indication of the abnormality is blood

dripping from the mouth. In a few cases the site of the hemorrhage is the socket of a loosened tooth. It is not uncommon to observe bleeding from the defective animal's nose but it is difficult to determine the source. Hemorrhage and swelling in the joints also occur, especially when the animals are kept on a concrete floor. Other regions subject to bleeding are: 1, ears, snout, and tail; the result of minor injury. 2, navel; hernia cases. 3, vagina; following coitus or parturition. 4, intestinal wall; may result from injury by intestinal parasites. 5, rectum; producing bloody stools. According to Mills (7) the most common sites of spontaneous hemorrhage in hemophilia are from the mouth or nose and into the joints.

Quick (8) devised a method for the diagnosis of hemophilia in man and set up certain criteria for the blood of a true case of hemophilia. In order to apply Quick's criteria to our animals five bleeder and five normal swine were tested by his methods, and our data are shown in table 3. The parallelism is close though the swine seem to be more mildly affected than the hemophiliacs.

A considerable number of valuable experimental animals have died from loss of blood, therefore an attempt was made to find a therapeutic measure that would reduce the coagulation time of the blood and arrest the hemorrhage. The blood-globulin fraction, prepared from normal swine blood by the method of Pohle and Taylor (9), has been very useful. It is our practice to inject, intramuscularly or intravenously, 2 ml. of a saline solution containing 5 to 10 mgm. of dried globulin substance for each pound of live weight. The whole-blood coagulation time, or fibrin-precipitation time, were not reduced to the normal range but in practically every case they were markedly lowered and the flow of blood was arrested. Typical examples are summarized in table 4.

SUMMARY

1. A strain of swine was encountered in which the blood clotting mechanism is defective.

2. The fibrin precipitation time in diluted plasma is a reliable test for the abnormality.

3. The characteristics of the disease fit very closely the criteria set up by Quick for the diagnosis of hemophilia.

4. The injection of a globulin fraction prepared from normal blood reduces the coagulation time of the blood of abnormal animals.

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THE ANOMALY OF A NORMAL DUKE'S AND A VERY PROLONGED SALINE BLEEDING TIME IN SWINE SUFFERING FROM AN INHERITED BLEEDING DISEASE[1]

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Hogan, Muhrer and Bogart (1) have described a bleeding disease in swine that is transmitted by both sexes as a Mendelian recessive (2). Examination of the blood of affected animals (1) shows normal concentrations of prothrombin, calcium, and fibrinogen; whole blood coagulation time is prolonged, and Duke's bleeding time is normal. Studies on the platelets indicate that they are abnormally stable, though present in normal number.

In a search for additional differences, we have found that affected animals have a prolonged Doettl and Ripke (3) bleeding time. In order more accurately to evaluate this anomalous finding, bleeder animals and suitable control animals have been tested at weekly intervals for a period of 3 months with both the Duke and the Doettl and Ripke methods. The results show a marked difference in the response of the affected animals to the two bleeding time tests.

METHODS. *Doettl and Ripke's bleeding time.* A modification by Copley and Lalich (4) was used. A lancet wound near or on the edge of the shaved ear to a depth equivalent to that used for Duke's test (1–2 cm. blot in 30 sec.) was considered acceptable. Care was taken to avoid small veins. The wound was immersed in 0.9 per cent NaCl solution brought to 37° in a 1 liter beaker. The bleeding time was the time that elapsed between lancet puncture and the cessation of any visible flow of blood from the wound. Duke's bleeding time: A lancet wound was made on the edge of the shaved ear and the blood was blotted at half-minute intervals with coarse filter paper until the flow had ceased.

The animals chosen for the 3 month experiment are described in table 1. All were purebred Poland Chinas belonging to one of 3 inbred lines of swine (lines 1, 2 and 3). Female 74 (line 1) was normal, but had produced bleeder offspring when bred to a bleeder boar. The other controls were from lines 2 and 3, inbred lines in which the bleeding disease has never been observed.

RESULTS. A summary of the weekly tests is given in figure 1. The curves on the left show that Doettl and Ripke's bleeding time is prolonged consistently in the affected animals. In normal animals bleeding stops promptly, usually in less than 100 seconds. The average bleeding time is 443+ seconds in the bleeders and 74 seconds in the normals for the 3 month period. There is therefore more than a 6-fold prolongation in the bleeders. The test seems to indicate those periods during which the affected animals are in greatest danger of hemor-

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TABLE 1

Description of animals used in the three month experiment

BLEEDERS	AGE	WEIGHT	NORMALS	AGE	WEIGHT
	mos.	*lbs.*		*mos.*	*lbs.*
F80	18	354	F74	18	342
F126	6	195	F68	6	203
			F139	6	229
M1	16	400	M81	18	570
			M30	7	276

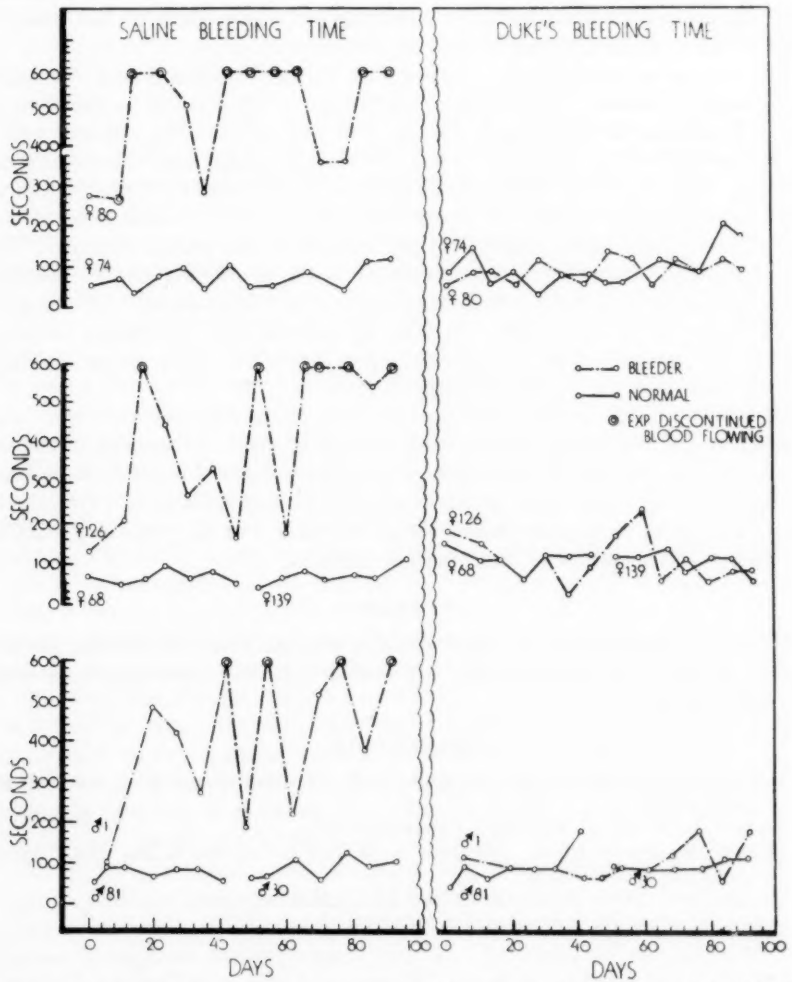

Fig. 1. A comparison of Duke's, and Doettl and Ripke's method, of determining bleeding time.

rhage. Thus, female 126 had a severe mouth hemorrhage on the 17th day. The saline bleeding time was over 10 minutes. On the 55th day, male 1 had a saline bleeding time of more than 10 minutes, and shortly thereafter almost bled to death from a superficial cut in the floor of his mouth. Both animals required special treatment (thrombin and pressure) to stop the flow of blood. Throughout the observations hemorrhages were observed repeatedly from the gums of the 3 bleeders at the points that came in contact with the rope used to tie them up. Mouth hemorrhages were never observed in the normal controls.

Readings were made on the bleeders for a maximum period of 10 minutes and in about 50 per cent of the tests blood was still flowing when the experiment was discontinued. We have not determined the upper limit of the bleeding time because of the difficulty in keeping the animals quiet.

The curves on the right (fig. 1) show that Duke's bleeding time is normal in the affected animals. The average Duke's time is 103 seconds in the bleeders and 102 seconds in the normals for the 3 month period. Six bleeders and 7 normal animals whose ages vary from 4 to 6 months, and in addition the animals listed in table 1 (except F68 and M81), have been tested by Duke's method, using Kleenex tissue instead of coarse filter paper. The average Duke's time was 173 seconds in the bleeders, and 195 seconds in the normal animals. The use of a highly absorbing paper apparently tends to prolong Duke's bleeding time, due perhaps to the more effective removal of surface blood. The lengthening occurs to the same extent in both the normal and the bleeder animals, however. The average saline time was 446+ seconds in the 6 young bleeders, and 77 seconds in the 7 young control animals.

Discussion. The saline bleeding time is a comparatively new test, and only a few observations have been made by that method. According to Copley and Lalich (4) the average in normal men is 95 seconds. No values have been published for the saline time in the human hemorrhagic diathesis. In view of our findings with swine, the clinical use of the saline test may prove of value in the further classification of the bleeding diseases.

SUMMARY

The saline bleeding time in swine suffering from an inherited bleeding disease is about 6 times the normal value. In contrast, Duke's bleeding time is consistently normal.

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THE EFFECT OF BODY SIZE UPON ENERGY EXCHANGE IN WORK

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Winslow and Gagge (1941) have found that a large man and a small man are about equally efficient in performing work on a bicycle ergometer but that the large man has an advantage over the small man in dissipating the excess heat produced in the work. The present study consists of a comparison of the energy exchange of a large man with that of a small man in grade walking and running on a motor driven treadmill.

The men used as subjects in the study were *EU* age 22, weight 99 kgm., stature 184.5 cm., and *MT* age 24, weight 61 kgm. and stature 170 cm. They were both excellent athletes with thin waist lines and no excessive fat deposits. The experiments were performed in an indoor laboratory in midsummer with the men clad in trunks, socks and tennis shoes. Both were accustomed to outdoor activity in the heat. Subject *EU* was particularly well acclimatized to heat by having operated a lawn mower on the university grounds for several hours each day and by having pitched 1 to 3 baseball games each week. The men were compared in 3 experiments in which the dry bulb temperature of the room ranged from 31.1 to 32.3°C. and the humidity from 68 to 74 per cent (table 1). In experiment 1 each man walked on the treadmill for 75 minutes at 5.6 km. per hour on an 8.6 per cent grade, an intensity of work which raised the metabolism to about 7 times the basal level. In experiment 2 the men walked on the same grade at 6.7 km. an hour but subject *EU* was forced by approaching exhaustion to stop after 63 minutes of walking. In experiment 3 the men ran slowly at 10.5 km. an hour on the level which raised the metabolism 43 per cent higher than did the work of experiment 1. Subject *EU* was exhausted after 30 minutes of work in experiment 3. In experiment 4 the work was the same as in experiment 2 but the dry bulb temperature of the room was raised to 35°C., the humidity reduced to 44 per cent and 4 electric fans turned on the men during work to evaporate the sweat as completely as possible (table 1).

The following determinations were made in each experiment: 1. Metabolism at 10 to 20 minute intervals during work by collection of expired air and analysis of samples on the Haldane apparatus. 2. Heart rate recorded continuously by means of a cardiotachometer. 3. Rectal and skin temperatures at frequent intervals by means of thermocouples (Aldrich, 1928). 4. Weight loss by weighing the men before and immediately after work.

Results. In experiment 1 the large man (*EU*) was never able to attain a balance between heat production and heat loss and became exhausted after 75 minutes of work. His skin and rectal temperatures and his heart rate continued to rise throughout the experiment (fig. 1). In contrast the small man (*MT*) reached a steady state with less than 1°C. rise in rectal temperature and a heart

rate of 130. The metabolic rates of the two men during work were almost identical when related to weight but in relation to surface area *EU* was 21 per cent higher than *MT* (fig. 1). The heat accumulated during work may be estimated approximately from the rise in body temperature using 0.83 for the specific heat of the body. If heat accumulated and mechanical energy released by elevation of the body in walking up the grade are deducted from the total metabolism the approximate rate of heat dissipation may be estimated. From these estimations we find that *EU* dissipated heat during work at an average rate of 243 Cal./m². per hour or 5 per cent faster than the rate of 232 by *MT*. However if heat dissipation is related to weight the rate of *EU* is 16 per cent lower than that of *MT*, the average values being respectively 5.5 and 6.4 Cal./kgm. per hour for the two men. Thus loss of heat was determined largely by the surface area and heat production by the body weight. The data in table 2 show that weight loss during work, due to sweating and loss through

TABLE 1

Average room temperature during the work experiments

	SUBJECT	DRY BULB	WET BULB
		°C.	°C.
Expt. 1	*EU*	31.7	27.4
	MT	32.2	27.4
Expt. 2	*EU*	31.7	27.8
	MT	32.2	27.3
Expt. 3	*EU*	31.1	26.7
	MT	31.6	27.0
Expt. 4	*EU*	34.8	24.9
	MT	35.2	25.2

TABLE 2

The average rate of weight loss during work

	SUBJECT	DURATION MIN.	WT. LOSS IN G. PER HR.	
			Per kgm.	Per m.²
Expt. 1	*EU*	75	25.4	1120
	MT	75	20.1	731
Expt. 2	*EU*	63	25.2	1120
	MT	71	26.2	920
Expt. 4	*EU*	74	20.1	884
	MT	74	20.2	725

respiration, was considerably greater in *EU* than in *MT* both in relation to surface and to weight, the difference being associated with the observed differences in skin and rectal temperatures. Under the humid conditions of the experiment each subject was wet with sweat within a few minutes after he started work and sweat began to drip off onto the treadmill. This led us to believe that both subjects had as great a rate of evaporation as air conditions permitted. Under the existing conditions the slightly greater rate of heat elimination by *EU* in relation to surface area was due to his higher skin temperature which produced greater radiation (fig. 1).

The results of experiment 2 substantially confirm those of experiment 1. Subject *EU* was forced to stop after 63 minutes of work with skin and rectal temperatures of 37.6 and 40.7°C. respectively and a heart rate of 180 while *MT* was able to attain heat balance with a rise of only 1.1°C. in rectal temperature (fig. 2). Because the rate of walking was greater in this experiment metabolism

was on the average about 22 per cent higher than in experiment 1 (fig. 2). The averages were 9.88 and 9.72 Cal./kgm. per hour for *EU* and *MT* respectively, and 440 and 346 Cal./m.^{2} per hour. After deducting heat accumulated and the energy of work as described above the average rates of heat dissipation were 6.15 Cal./kgm. per hour for *EU* and 7.60 for *MT*, a difference of 23 per cent. As in experiment 1 the rate of heat elimination was closely related to surface area the mean values being 274 and 271 Cal./m.^{2} per hour respectively for the two men. Weight loss in *EU* was at the same rate as in experiment 1 which was apparently very close to his maximal rate of sweating because on the basis of skin and rectal temperatures the stimulus was considerably greater in experiment 2 (table 2). *MT* sweated faster in the second experiment than in the first though still less than *EU* in relation to surface area. These rates of sweating are as great as the highest records of Robinson, Dill, Wilson and Nielsen

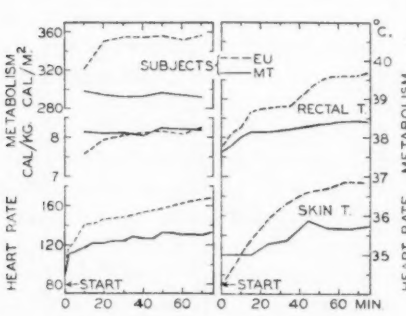

Fig. 1

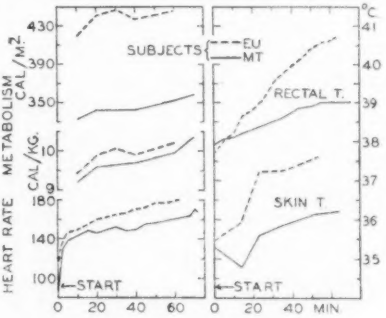

Fig. 2

Fig. 1 Records of body temperature, metabolism and heart rate of the subjects during experiment 1.

Fig. 2. Records of body temperature, metabolism and heart rate of the subjects during experiment 2. *EU* was exhausted and stopped after 63 minutes of the walk.

(1941) on a southern Negro performing similar work in a warm, humid environment.

The work and the environment in experiments 1 and 2 imposed conditions which overtaxed the capacity of *EU* to regulate his body temperature. With environmental conditions the same a further increase in the intensity of work was used in experiment 3 with the idea of reaching the maximal capacity of *MT* for temperature regulation. The results of the experiment are shown in figure 3 and reveal that even though his metabolic rate was 20 per cent higher than in experiment 2 and 43 per cent higher than in experiment 1 *MT* was able to approach a steady state in metabolism, rectal temperature and heart rate. He maintained the work for 40 minutes and apparently could have gone on much longer whereas *EU* was forced to stop after 30 minutes of work with a rectal temperature of 40.5°C. and a heart rate of 182. The metabolic rate of *EU* in relation to weight was only 6 per cent higher than that of *MT* at the start of

work but as his temperature rose during the 30 minutes of work the difference increased gradually to 23 per cent (fig. 3).

In experiment 4 the men worked at the same rate as in experiment 2 but the humidity was lower and fans were used to evaporate the sweat. The room temperature was higher than skin temperature after work started (fig. 4) therefore evaporation was the sole mechanism of heat dissipation. Each man attained heat balance and a steady state of heart rate during the experiment (fig. 4). The rectal temperature of *EU* reached a plateau 1.7°C. below his final temperature in experiment 2. He was slightly more efficient than *MT* and did not have an increasing metabolic rate during the progress of the experiment as in the preceding experiments this being associated with the fact that his body temperature did not increase so greatly as before. Skin temperatures of both men during work dropped markedly below dry bulb air temperature because the air movement and low humidity almost completely evaporated the sweat. It

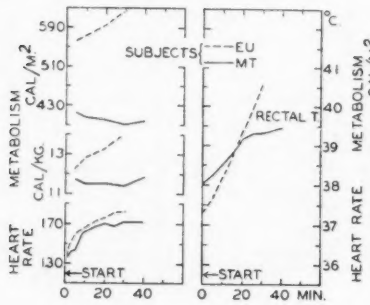

Fig. 3

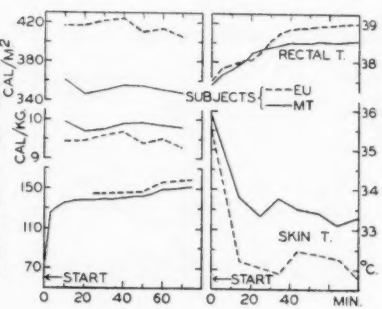

Fig. 4

Fig. 3 Records of rectal temperature, metabolism and heart rate of the subjects during experiment 3. *EU* was exhausted and stopped after 30 minutes of the run.

Fig. 4 Records of body temperature, metabolism and heart rate of the subjects during experiment 4.

may be observed in figure 4 that the skin temperature of *EU* dropped to an average of 32.1°C. after work began or 1.5°C. lower than the average of *MT* during work. This difference between the two men is accounted for by the fact that the water loss of *EU* was 884 g./m.² per hour and that of *MT* was only 725 (table 2). Since his skin temperature was lower *EU* dissipated 310 Cal./m.² per hour as compared with 277 by *MT*. The rate of sweating in relation to body weight was the same in the two men (table 2), a similarity which depends upon the fact that the total heat production was closely related to weight and the dissipation of this heat depended upon evaporation.

Discussion. Winslow and Gagge (1941) found that the efficiencies of a large man and a small one in work performed on a bicycle ergometer were equal and that the increase in metabolic rate during work was not related to body weight but was proportional only to the work output on the ergometer. This

is true in this type of work because the subject does not have to move his body about in accomplishing the work. Since the large man's total surface area is greater and his total increase in heat production the same as the small man's for a given work output on the ergometer the large man can dissipate the extra heat more effectively.

The two subjects used in this study, a large man and a small one, were equally efficient in grade walking on the treadmill and since the work consisted entirely of moving and elevating the body the metabolic rates of both men during work at a given speed and grade were closely related to body weight. When the rate of walking was increased the further increase of metabolic rate was proportional to the body weight. The two subjects were greatly different in the ratio of weight to surface area the large man having 44 kgm. of weight per m.^{2} of body surface as compared with 35 in the small man. Thus when both men walked at the same rate and heat production was proportional to weight the large man produced in relation to surface area about 20 per cent more heat than the small man. For this reason in these experiments where a warm humid environment restricted heat dissipation the large man was forced to store heat throughout periods of work in which the small man could easily attain heat balance. Similar differences would be found in such types of work as climbing, shoveling or even bicycling on the road where all or a large part of a man's work output consists of moving his body about. The army, as well as industry, might well improve efficiency on some jobs if these factors were considered in selecting personnel for work in hot environments. The important factors to be kept in mind are the ratio of body weight to surface area, the type and difficulty of the work, and the temperature and humidity of the environment.

SUMMARY

Two men ran or walked up a grade on a motor driven treadmill at rates which raised metabolism 7 to 11 times the basal level. One of the subjects weighed 99 kgm. and had 44 kgm. of weight per square meter of body surface and the other weighed 61 kgm. and had 35 kgm. per square meter. The efficiencies of the two men in performing the work were the same. In work which raised metabolism to 8 Cal./kgm. per hour, when heat dissipation was limited by a room temperature of 32°C. and a humidity of 70 per cent, the larger man accumulated heat throughout the work and was forced to stop with approaching heat exhaustion after 75 minutes. Under the same conditions the smaller man attained heat balance and a steady state of heart rate at a metabolic rate of 9.7 Cal./kgm. per hour. Both men in this type of work produced heat in proportion to body weight and since the ratio of weight to surface area was 20 per cent greater in the large man than the small man his heat production in walking at a given rate when related to surface was about 20 per cent greater. Therefore, since heat dissipation depended largely upon the skin surface, at rates of walking in which the small man could easily maintain heat balance under these environmental conditions the large man could not dissipate all of his body heat.

Acknowledgment. The author is greatly indebted to Eugene S. Turrell for technical assistance in the study and to Melverne I. Trutt and Emil Uremovich who liberally donated their time and efforts to act as subjects in the experiments

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THE REACTION AND NEUTRALIZING ABILITY OF THE CONTENTS OF THE FIRST PART OF THE DUODENUM IN NORMAL DOGS UNDER FASTING CONDITIONS[1]

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The contents of the first part of the duodenum in normal dogs display an ability to neutralize, buffer and dilute gastric chyme which varies with the type of foodstuffs undergoing digestion, but which, nevertheless, is considerable (3, 4, 5). This study was undertaken in an attempt to determine whether a neutralizing efficiency obtained in the duodenal bulb under standardized conditions of fasting similar to that observed after different meals. Through the simultaneous determination of the various criteria of acidity in the pyloric antrum and duodenal bulb, we hoped to learn whether the physiologic relationships affecting the acidities in these contiguous areas were in any way modified from those observed during digestion under similar experimental conditions.

METHOD. Dogs were prepared with cannulated gastric and duodenal fistulas (7, 8) and then trained and maintained in the manner described previously (3). For a period of 36 hours preceding each experiment, and in a few instances for a period of only 24 hours, all food was withheld but water was permitted as desired. Each dog was placed on a table supported by a muslin hammock in a position which it had been trained to maintain. Following the insertion of the collecting and instillation tubes, during which process care was taken to cause as little trauma as possible, a rest period of 30 minutes was allowed for any unavoidable excitement or traumatic stimulation to subside. At the end of this period fractional samples were removed simultaneously from the pyloric antrum and the duodenal bulb at 10 minute intervals for ½ hour. As a rule all the material which could be obtained was collected at each aspiration.

The pH was determined on each gastric and duodenal sample without delay through the use of a Leeds-Northrup pH indicator which employs a glass electrode. The free and total acidity of each specimen was titrimetrically estimated after filtration, using Toepfer's reagent (dimethyl-amino-azobenzene) and phenolphthalein as the respective color indicators (2). On each duodenal sample, in addition, there was determined what was named the excess neutralizing ability. This consisted of the amount of N/10 hydrochloric acid which was required to cause a positive colorimetric reaction for free acid (2, 3).

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Portion of thesis submitted by Doctor Berk to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Medical Science (D.Sc.(Med.)) for graduate work in internal medicine.

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In all, 77 technically satisfactory experiments were performed on 7 dogs. These totaled 1437 different determinations which included 429 estimations of pH, 409 determinations of free acid, 399 determinations of total acidity and 200 estimations of excess neutralizing ability of the duodenal contents.

RESULTS. The quantity of material obtained from the stomachs and duodenums of the fasting dogs was variable. As a rule that gotten from the stomach was small, at times too small to make satisfactory pH readings. Duodenal secre-

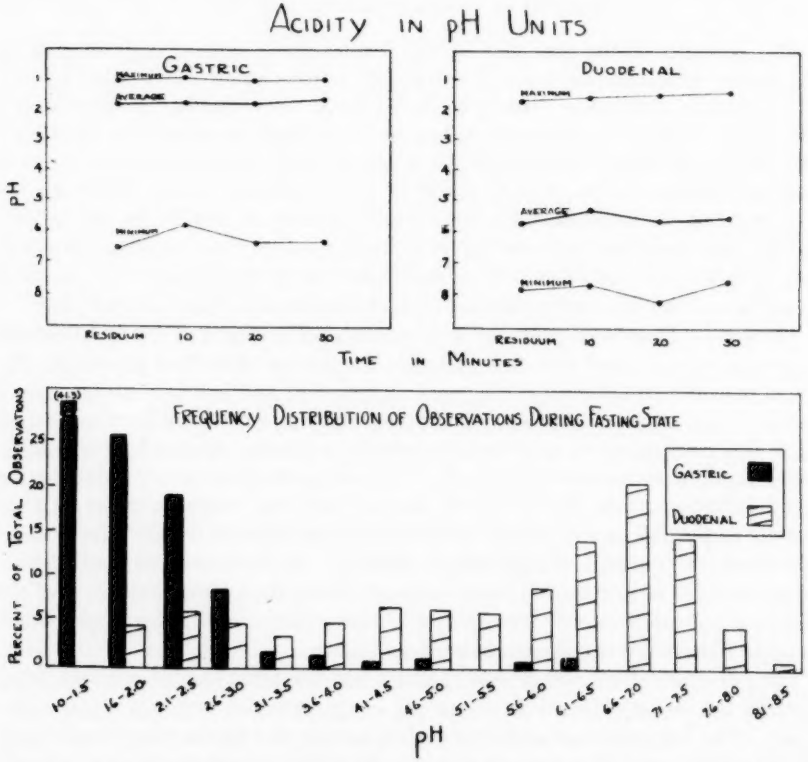

Fig. 1. Acidity in pH units of samples collected simultaneously from just above and just below the pylorus in fasting normal dogs.

tion, on the other hand, while far from profuse was usually greater in quantity than that obtained simultaneously from the stomach. Both the volume and reaction of the stomach contents were rather constant for each dog although individual variations were seen.

Acidity in pH units (fig. 1). *Stomach.* The average pH of the fasting gastric contents was fairly constant even though the individual specimens showed a wider range of variation (pH 0.99 to pH 6.5) than that observed following the use

of various gastric secretory stimuli. The average pH value during the fasting observation period (pH 1.90) was greater than that during the post-Ewald meal period (pH 1.63), but was decidedly less than those during the post-Liebig's extract-histamine (pH 2.01) and post-cream meal (pH 3.40) periods.

Duodenum. A very slightly changing average pH and a wide range of variation in the pH of the individual samples (pH 1.67 to pH 8.14) were also characteristic of the contents of the duodenal bulb. Fluctuation in the pH values of consecutive specimens was much more apparent than during the active digestion of various foodstuffs.

The ability of the contents of the first part of the duodenum to rapidly neutralize, buffer and dilute the secretions received from the stomach was as good, and in some respects superior, to that displayed during periods of digestion. A difference in pH was constantly maintained between the simultaneously obtained contents of the pyloric antrum and duodenal cap. Throughout the period of observation in the fasting state this averaged 3.66 pH units. Of all the fasting duodenal specimens 17.2 per cent had a pH of 3.5 or less, the value which we had adopted as the critical one for free acid (2). This percentage is just about the same as that after an Ewald meal (16.4 per cent) (3); it is less than that after a protein-histamine meal (26.5 per cent) (4); but it is greater than that after a fat meal (1.1 per cent) (5). On the other hand, the average fasting duodenal pH value was higher than the average pH value after any of the foodstuffs (fasting, pH 5.56; cream meal, pH 5.51; carbohydrate-water meal, pH 4.82; meat extract-histamine meal, pH 4.54).

Free acid (fig. 2). *Stomach.* The gastric free acid values in the fasting state closely simulated those following carbohydrate or meat extract-histamine stimulation both in average value and in range of distribution; they definitely exceeded the values obtained after the gastric secretory inhibitor, cream. The percentage of specimens colorimetrically negative for free acid (4.3 per cent) was not greatly in excess of the equivalent percentage after an Ewald meal (0.9 per cent) but was much less than after cream (53.2 per cent) or beef extract-histamine (18.6 per cent) meals.

As in the case of pH, the average gastric free acid values were fairly constant while wide fluctuations in individual values from determination to determination were not uncommon.

Duodenum. As evidence of the ability to rapidly neutralize the gastric secretion, the contents of the first part of the duodenum in the fasting state characteristically showed an absence of free acid. The percentage of samples colorimetrically positive for free acid (6.3 per cent) was less than the percentage (17.2 per cent) electrometrically positive (pH 3.5 or less) (fig. 1). This is attributable to the error introduced by our method of filtration and dilution preparatory to colorimetric titration which results in a number of false negative readings (2). The percentage of colorimetric free acid-positive duodenal samples, similar to the electrometric percentages, is nearly equal to that in the post-Ewald meal phase (2.6 per cent), and is less than that after beef extract and histamine (12.2 per cent). No free acid was observed colorimetrically after cream.

A nearly quantitative constancy was again displayed in the average values for duodenal free acid.

Total acidity (fig. 3). *Stomach.* The average gastric total acidity values were a little less than those after the more potent secretagogues but were greater than those following a fat meal. The range of distribution of the values was as wide as with any of the meals, although the averages were fairly constant.

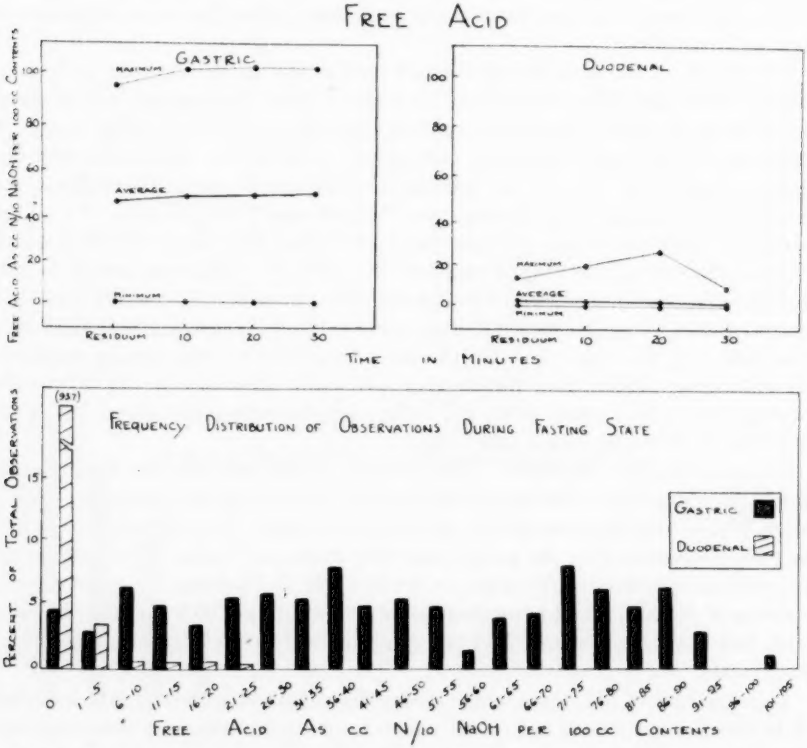

Fig. 2. Free acid as determined on samples collected simultaneously from just above and just below the pylorus in fasting normal dogs.

Duodenum. The efficient neutralizing mechanism in the duodenal bulb in the fasting state is evidenced again by the fact that the average value for total acidity in the duodenum during the entire period of observation (22 clinical units) was definitely less than comparable values for the entire post-meal phase following the various foodstuffs (carbohydrate-water, 30 clinical units; meat extract-histamine, 36 clinical units; cream, 47 clinical units). The range of the individual values was not as wide as during the digestive intervals with the exception of that after the Ewald meal. The average values, as in the stomach, were fairly constant.

Excess neutralizing ability of the duodenal contents (fig. 4). The excess neutralizing ability of the duodenal bulb contents has been defined as a measure of the reserve capacity which these contents possess to neutralize, buffer and dilute the material received from the stomach above that necessary to offset the free acid content (2, 3).

Both in the average value throughout the observation period and in the range of distribution of the values, the average excess neutralizing ability of the

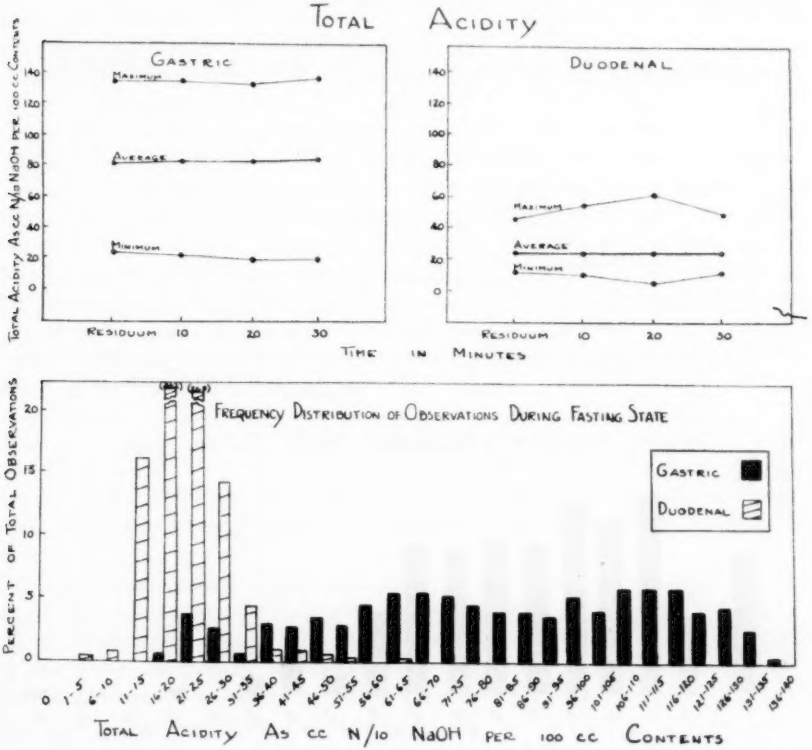

Fig. 3. Total acidity of samples collected simultaneously from just above and just below the pylorus in fasting normal dogs.

duodenal contents compared well with that seen following the use of different foodstuffs. In these respects it closely resembled the findings after the Ewald meal, a tendency which was noted with all the other types of determinations.

Of all the observations in the fasting state, 8.0 per cent failed to show (colorimetrically) any excess neutralizing ability, whereas 3.0 per cent of the post-Ewald meal and 16.6 per cent of the post-Liebig's extract-histamine samples were similarly negative; all of the post-cream meal specimens showed some excess neutralizing capacity.

Discussion. Many normal and abnormal stimuli sufficient to influence gastric secretion may have existed in our dogs (1) despite our attempt to avoid as many of them as possible and to secure a true basal state. Consequently, the quantitative values for fasting gastric acidity which we obtained are not to be interpreted as necessarily normal values. Nothing, however, militates against their usefulness in affording a comparison with similar values simultaneously obtained in the first part of the duodenum or with corresponding values obtained in the digestive state under similar experimental conditions.

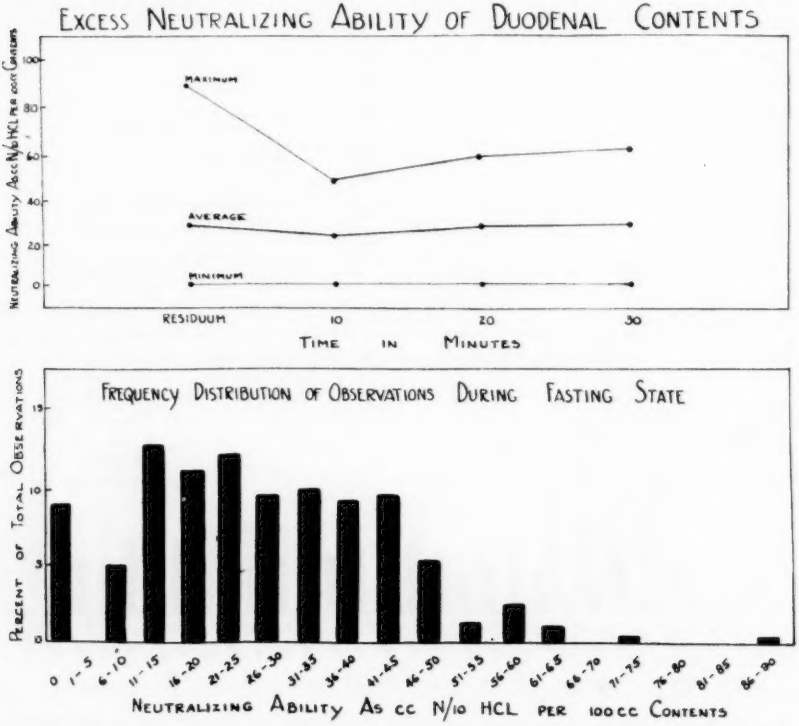

Fig. 4. Excess neutralizing ability of the duodenal contents in fasting normal dogs

Although variations occur, nevertheless, the volume and the acidity of the stomach contents in the fasting normal dog appear to be more or less characteristic for each animal just as various gastric secretory stimuli produce responses which are fairly characteristic for each.

A striking feature of the fasting state is a tendency for consecutive observations to fluctuate widely in value both in the stomach and duodenum. As a consequence, the pH values, in particular, vary through a range that is wider than that observed during periods of digestion. In spite of the fluctuation of the individual readings, the average values for all of the several determinations ex-

hibit an arresting uniformity. These characteristic tendencies for the values to show wide individual fluctuation but aggregate relative constancy are the most prominent features which distinguish fasting observations from those made during the active digestion of various foodstuffs.

The contents of the first part of the duodenum of normal fasting dogs display an ability to neutralize, buffer and dilute the gastric secretions which is equal to, and in some respects even surpasses, that seen during the digestive state. A difference in reaction is constantly maintained between the contiguous pyloric antrum and duodenal bulb which, on the average, is greater than that during digestion. That effective neutralization (ability to neutralize gastric free acid manifested by the ability to maintain a pH of 3.5 or above) is well maintained is shown by the over-all average duodenal pH of 5.56 which is higher an over-all average pH than that following various meals; still, approximately 1 out of every 6 (17.2 per cent) of the duodenal samples contained free acid (pH 3.5 or less).

It is of interest to note that the average hydrogen ion concentration in the stomach of fasting normal dogs is much greater than that in normal fasting people studied in an analogous manner (pH 1.90 in dogs; pH 3.51 in people) (6). The average duodenal hydrogen ion concentrations, in contrast, are about equal (pH 5.56 in dogs; pH 5.60 in people). It may well be that this superior neutralizing capacity in the duodenal bulb of dogs in part underlies the rarity of spontaneous duodenal ulcers in dogs on the one hand, and the frequency of this disease in humans on the other.

SUMMARY AND CONCLUSIONS

1. The contents of the first part of the duodenum of normal fasting dogs display an ability to neutralize, buffer and dilute the gastric secretions which is equal to, and in some respects even surpasses, that seen during the digestive state.

2. The volume and acidity of stomach contents in the fasting normal dog appear to be more or less characteristic for each animal.

3. A tendency for the various criteria of acidity in the stomach and duodenum to show wide individual fluctuation but aggregate constancy are the most prominent features which distinguish fasting observations from those made during the active digestion of various foodstuffs.

4. The over-all average pH in the stomach of fasting normal dogs (1.90) is much less than that in fasting normal people (3.51); the over-all average pH values in the duodenal bulb, nevertheless, are about equal (dogs, pH 5.56; people, pH 5.60).

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HYPOTHALAMIC STIMULATION YIELDING ADRENALIN REVERSAL EFFECTS

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It has been shown that both pressor and depressor effects may be obtained on hypothalamic stimulation. While sympathetic and parasympathetic vascular responses have been attributed to different areas of the hypothalamus (Beattie, 1932), Kabat, Ranson and Magoun (1935) did not find a definite anatomical subdivision of vasopressor and depressor reactions. Hare and Geohegan (1939) noted that variations of frequency of stimulation altered the blood pressure response. Bronk, Pitts and Larrabee (1941) stressed the importance of afferent impulses and the pre-existing blood pressure level on the nature of the sympathetic discharge obtained on stimulation of the hypothalamus. While much attention has been directed toward the type and location of the neural response, there has been comparatively little consideration of the humoral aspects. In this investigation we wish to point out that adrenalin released by hypothalamic stimulation is an important factor in the determination of the character of the resultant blood pressure curve in the cat. The completely denervated iris was used as an indicator of circulating adrenalin. The pupil of such a preparation in the cat is very sensitive and dilates with intravenously injected adrenalin in concentrations of 1×10^{-8} per kilo of body weight (Bender and Weinstein, 1940).

METHOD. Cats and monkeys (Macaca mulatta) were used in these experiments. The iris was completely denervated (C.D.) by sectioning the optic and all ciliary nerves behind the globe. The arterial tension was recorded from a cannulated femoral artery. The animals were mounted in the Horsley-Clarke apparatus and the hypothalamus stimulated with a bipolar electrode after the technic described by Ranson (1934). Current was supplied through a thyratron condenser circuit. The frequency range used was between 12 to 250 per second. All stimulations were carried out under light nembutal anesthesia. In many instances the anesthesia was so light that when the stereotaxic apparatus was removed the animal appeared to be almost fully awake.

OBSERVATIONS. a. *Hypothalamic stimulation in the cat.* Two types of blood pressure response were observed—neural and humoral. The neural reactions were characterized by an abrupt rise in arterial tension which occurred after a latency of 1 to 2 seconds. At times the increase was as much as 100 mm. Hg. Paralleling this pressor response was a prompt dilatation of the normal pupil and retraction of the normal nictitating membrane (N.M.). These were parts of a sympathetic neural response. The humoral effect appeared 8 to 15 seconds

[1] Aided by grants of the Josiah Macy, Jr. Foundation and the Dazian Foundation for Medical Research.

after the application of the stimulus and was characterized by a slow dilatation of the c.d. iris to the maximum diameter. This was a consistent and spectacular phenomenon. The increase in the pupillary diameter varied from 1 to 6 mm. depending on the initial status; the maximal diameter was 13 or 14 mm. The mydriasis persisted for over 60 seconds after the cessation of the stimulus. Simultaneous with the onset of the mydriasis in the c.d. iris, the blood pressure began to fall as much as 40 mm. Hg. There was no apparent change in the pulse rate. The blood pressure continued to drop during the stimulation period, which was usually for 60 seconds. However, the normal pupil remained dilated, the nictitating membranes retracted, and the pupil in the c.d. iris maximally dilated. When the stimulus was removed, the normal pupil and the nictitating membrane quickly returned to prestimulatory status, while the pupil of the completely denervated iris remained at maximal dilatation, returning slowly to its initial diameter after 90 to 120 seconds. With cessation of stimulus, the fall in blood pressure was often accelerated and a deep dip below the base line resulted. The depressor effects were never accompanied by bradycardia, were not blocked by atropine and were not potentiated by eserine.

These effects were best produced with frequencies ranging from 40 to 250 per second. They could be obtained by stimulation of points throughout the entire hypothalamus in both anterior and posterior divisions. The reactive foci were situated in the region surrounding the third ventricle and were most consistently seen at coördinates 1 to 4 mm. lateral to the midline and from 6 to 8 mm. below the zero horizontal plane. The more lateral hypothalamic areas, though yielding good neural effects, were less productive of humoral responses. In other instances, the initial pressor action was not observed; instead there was an initial drop in blood pressure, retraction of the nictitating membrane, dilatation of normal pupil and after the usual latency of 8 to 15 seconds, a further drop in the arterial tension this time accompanied by dilatation of the pupil in the c.d. iris. In several animals, drops in blood pressure occurring 3 to 5 seconds after the start of the stimulus were obtained, but these were not accompanied by mydriasis in the denervated iris and were considered to be neural effects.

b. *Effects of adrenalectomy.* Unilateral adrenalectomy did not alter the results of hypothalamic stimulation but bilateral adrenalectomy or acute section of the thoracic spinal cord abolished the delayed mydriatic effects and almost completely obliterated the delayed drop in blood pressure. The disappearance of the delayed vasodepression was not a consistent phenomenon. In some instances the fall in blood pressure though not pronounced was found even after bilateral adrenalectomy. The pupil in the c.d. iris did not dilate even when very strong current was used. The immediate abrupt rise in blood pressure, the dilatation of the normal pupil and the retraction of the normal nictitating membrane were preserved and maintained during the period of stimulation. In some cats in which there was no initial pressor effect, hypothalamic stimulation after bilateral adrenalectomy had no influence on the blood pressure level, the curve remaining almost flat.

c. *Effects of adrenalin.* Some of these findings suggested that the stimulation of secretions from the adrenal glands were responsible for some of the delayed fall in arterial tension. Further proof was found when low concentrations of saline extract of the excised adrenal gland were injected intravenously into the adrenalectomized cat. These reproduced the humoral response obtained on hypothalamic stimulation, i.e., 1, a drop in blood pressure, and 2, a dilatation of denervated iris six to eight seconds after the intravenous injection. In order to quantitate these results, commercial adrenalin (Parke-Davis and crystallin form of Winthrop) was used. It was found that 0.01 gamma per second per kilogram of body weight injected intravenously for 3 to 5 seconds in a 1 cc. volume duplicated the humoral effects of electrical stimulation of the hypothalamus. Simultaneous stimulation of the hypothalamus and intravenous adrenalin injections in the above concentrations produced in the adrenalectomized cat the same pupillary responses and blood pressure curves as in the normal. Larger doses of adrenalin yielded only pressor effects. No delayed pressor effects coming on eight or more seconds after the onset of stimulus were observed. The delayed depressor response was neither blocked by atropine nor enhanced by eserin.

d. *Hypothalamic stimulations in the monkey.* In the monkey the synchronous delayed mydriatic and depressor effects were not observed. Frequent depressor as well as pressor reactions and pupillary dilatation in the normal eye were obtained, but the vascular responses were apparently neural. In contrast to the cat, conspicuous pupillary "humoral" effects were rarely seen. On one occasion, a slight mydriasis was noted in the denervated iris after an intramuscular injection of cocaine. In another monkey delayed mydriasis was obtained under urethane anesthesia. Adrenalin blood pressure reversal effects were not observed. Doses of 0.10 to 0.01 gamma of adrenalin injected intravenously produced a rise in blood pressure. Smaller doses had no effect on the arterial tension.

Discussion. The above experiments confirm the fact that adrenalin is liberated on electric stimulation of the hypothalamus in the cat as previously demonstrated by Houssay and Molinelli (1925), Beattie, Brown and Long (1930). Magoun, Ranson and Hetherington (1937) used the sympathetically denervated nictitating membrane as the indicator for adrenalin in similar experiments. From our own experience we find the nictitating membrane, following excision of the superior cervical ganglion, is not nearly as reliable an indicator for adrenalin as the c.d. iris possibly because the membrane in such a preparation is only partially denervated. Adrenalin not only affects the pupil but also influences the blood pressure curve. In small quantities intravenously injected adrenalin was shown by Elliot and Durham (1906) to produce a drop in blood pressure. It is not surprising, therefore, to note delayed vaso-depressor effects on hypothalamic stimulation. These depressions, along with the delayed mydriasis, are abolished by adrenalectomy.

The amount of adrenalin discharged on hypothalamic stimulations under nembutal anesthesia is probably on the order of 0.01 gamma per kilo per second.

This was estimated by determining the quantity of intravenously injected commercial adrenalin necessary to reproduce the mydriasis and fall in blood pressure obtained on hypothalamic stimulation. Such small quantities of adrenalin reproduce the syndrome of delayed mydriasis in the c.d. iris and a drop in the blood pressure. It is probable, however, if extremely strong electrical stimuli were applied, larger quantities of adrenalin might be liberated and the delayed depressor effect would be replaced by a pressor reaction. Houssay and Molinelli (1925) on stimulating the hypothalamus of the dog estimated 8 to 15 gamma of liberated adrenalin, which gave a marked and sustained rise in blood pressure. Stewart and Rogoff (1924) found that stimulation of one splanchnic nerve in the cat under ether anesthesia resulted in the secretion of adrenalin at the rate of 0.013 gamma per second per kilo of body weight. The amounts of adrenalin liberated during rage or struggle, however, are difficult to quantitate. In three of our experiments the stimulation of an adrenergic point in the hypothalamus with an ensuing delayed mydriatic and depressor response was accompanied by the so-called pseudoaffective rage components. When the anesthesia was very light, stimulation produced violent struggle, rhythmic panting, meowing, hissing, spitting, dilatation of the pupil in the c.d. iris, and surprisingly a drop of 10 mm. Hg in the blood pressure. We were unable to estimate the amount of adrenalin liberated during these stimulations but judging from the duration of the mydriasis in the c.d. iris it must have been more than 0.01 gamma per second per kilo.

It may be that in different emotional states, varying quantities of adrenalin are liberated, the larger yielding pressor and the smaller depressor effects in cats. It is also likely that more than one hormone is secreted during physiologic activation of the hypothalamus. Clark and Wang (1939) found evidence for the release of a pressor hormone from the posterior lobe of the pituitary, while Magoun, Ranson and Hetherington (1937) were able to detect a discharge of sympathin on electric stimulation of the hypothalamus in the cat.

SUMMARY

1. Hypothalamic stimulation in the cat produces simultaneously delayed depressor and mydriatic effects. These responses are humoral and are due to liberation of small amounts of adrenalin.

2. The dilatation of the pupil in the denervated iris and some of the delayed vaso depressions thus obtained can be abolished by adrenalectomy and reproduced by intravenous injection of adrenalin.

3. Some of the delayed falls in blood pressure are due to the liberated adrenalin—an adrenalin reversal effect.

4. Adrenalin reversal effects in the monkey could not be demonstrated either by hypothalamic stimulation or intravenous injections of adrenalin.

We are indebted to Mr. M. Grabiner for his technical assistance in this problem.

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THE INFLUENCE OF POSTURE ON BLOOD FLOW IN THE DOG[1]

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When subjects are placed in the upright position there is a marked retardation of blood flow in the lower extremities (1). This slowing appears to be due primarily to stagnation or pooling in the veins. The present study was designed to give further and more direct information concerning the blood flow changes with posture as measured by the thermostromuhr method in different parts of the body.

Sixty successful experiments were carried out on 20 dogs lightly anesthetized with chloralose (40 to 80 mgm. per K. B. W.) or Na barbital (180 to 250 mgm. per K. B. W.). They were placed in a trough which was rotated above a transverse horizontal axis, the position of which was adjusted in each case to coincide with the axis of the cannula used for recording the blood pressure from the carotid or the femoral artery. The distance from the point of arterial cannulation to the fourth interspace (heart level in the upright position) was measured and the value thus obtained applied as a correction for the hydrostatic pressure effect to all blood pressure readings made with the animal in the upright position. The animal was prevented from slipping when in the upright (75°) position by tying the mouth securely around a bit fixed to the animal board. Respiration was measured by means of a pneumograph. Blood flow was measured by means of direct current thermostromuhrs (2) in the femoral artery and vein, the renal artery and vein and the carotid artery and jugular vein. The units were connected to a high sensitivity Leeds and Northrup galvanometer through a double-throw switch which made it possible to make alternate (and practically simultaneous) observations of the flow in pairs of vessels or to study the flow in each vessel in successive experiments without disturbing the position of the thermostromuhrs. The units chosen for application to the vessels to be measured were of such size as to fit the vessels snugly and cause slight constriction. The usual procedure was to take a short control in the horizontal position after which the animal was tilted to an angle of 75°, feet down, and kept in this position for periods varying from 3 to 20 minutes before being returned to the horizontal. The tilting usually took about two seconds. Measurements of the flow were usually continued for at least 15 minutes after the animal was returned to the horizontal position. It was found impossible to obtain accurate readings of the changes in flow as the animal was tilted or immediately thereafter. This may have been due to rapid changes in temperature of the blood as a result of its sudden redistribution (3). Some difficulty was also experienced in obtaining

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venous blood flows when the respiration was very deep or fluctuated considerably. When this occurred, average readings were usually recorded. Considerable variation in the response to tilting was encountered due to the practical impossibility of achieving the same level of anesthesia in different animals as well as to variations in their compensatory respiratory and vasomotor mecha-

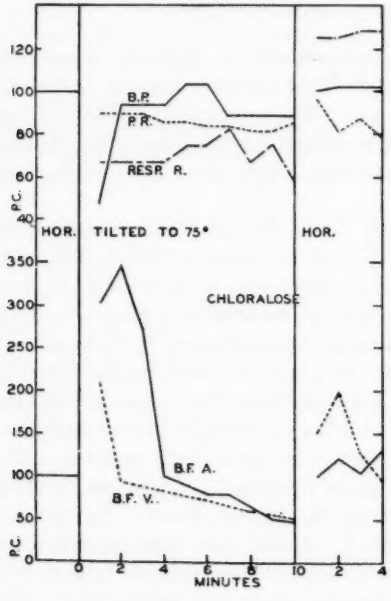

Fig. 1

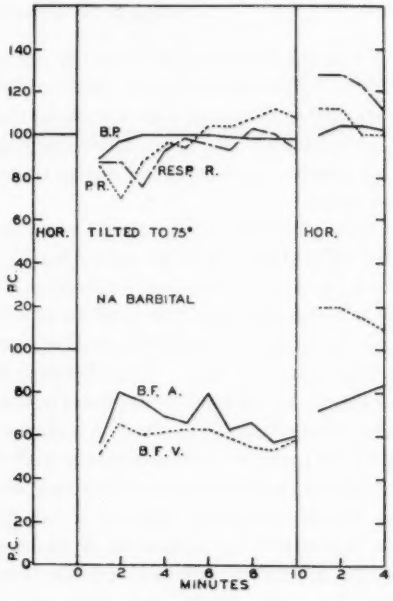

Fig. 2

Fig. 1. Percentage changes in blood flow in the femoral artery, *B.F.A.* and vein, *B.F.V.* Blood pressure, *B.P.*, recorded from opposite femoral artery and corrected for hydrostatic pressure effect. *P.R.* = pulse rate; *Resp. R.* = respiratory rate. Control values: *B.P.* = 145 mm. Hg; *B.F.A.* = 56 cc. per min.; *B.F.V.* = 42 cc. per min.; *P.R.* = 126 per min.; *Resp. R.* = 12 per min.

Fig. 2. Percentage changes in blood flow in the carotid artery, *B.F.A.*, and jugular vein, *B.F.V.* Blood pressure, *B.P.*, recorded from opposite carotid artery and corrected for hydrostatic pressure effect. *P.R.* = pulse rate; *Resp. R.* = respiratory rate. Control values: *B.P.* = 160 mm. Hg; *B.F.A.* = 67 cc. per min.; *B.F.V.* = 30 cc. per min.; *P.R.* = 267 per min.; *Resp. R.* = 14 per min.

nisms. This was particularly true of the changes for the first few minutes after tilting and after return to the horizontal position.

The ranges of blood flows in the various vessels were similar to those found by other investigators using the same or different methods (4). No differences were observed between the two anesthetics. With the animal in the horizontal position, control blood flows in cubic centimeters per minute in different experiments varied as follows: femoral artery—42 to 166; femoral vein—40 to 132;

carotid artery—23 to 189; jugular vein—40 to 148; renal artery—53 to 360; renal vein—44 to 248.

Typical changes in the rates of flow in the femoral artery and vein when the animal was tilted are shown in figure 1. The fall in intrafemoral pressure, evident immediately or within a minute after tilting, was usually associated with an increase in arterial and venous flow. Within several minutes, however, the rate of flow in the vein dropped sharply so that at the end of 10 minutes the rate of flow was only 12 to 64 per cent of the control rate before tilting. In several experiments the flow in the vein was almost stopped at the end of this period. The flow in the femoral artery showed similar changes except that the decrease usually came several minutes later and was never quite as marked as in the vein, the values at the end of 10 minutes being 50 to 84 per cent of the control rates. These results, indicating a slowing of the blood flow in the extremities, have been corroborated by determination of the oxygen saturation of the arterial and venous blood and the finding of a definite and often marked increase in the arterio-venous oxygen difference (5).

The rates of flow in the carotid artery and jugular vein decreased immediately after tilting and remained low except for a few instances where there was a secondary rise lasting from 3 to 5 minutes (fig. 2). At the end of 10 minutes the rates of flow had decreased to 50 to 90 per cent of the control rates in both vessels. These changes were usually related to those in carotid blood pressure and were accompanied by a marked decrease in oxygen saturation of the blood in the jugular vein (5). Loman and Myerson (6) reported similar changes in jugular blood flow in man.

The changes in flow in the renal artery and vein were less consistent. In the six experiments in which the rate of flow was measured in the renal artery, three showed results similar to those given in figure 3. In two experiments there was no significant change in blood flow over a ten-minute period while in the sixth experiment the rate of flow diminished with the assumption of the upright position but rose to the control value after 3 minutes. Likewise, results similar to those depicted in figure 3 (decrease to 30 to 40 per cent) were obtained in 3 of 4 experiments in which the rate of flow was measured in the renal vein. No changes were observed in the fourth experiment. In one experiment, renal vein flow diminished from 124 to 5 cc. per minute at the end of 10 minutes in the upright position. Smith (7) studying the diodrast and inulin clearances in man, found similar changes with posture. The failure of the filtration fraction (inulin clearance divided by effective plasma flow) to vary inversely with the renal blood flow was interpreted as indicating a constriction of afferent arterioles.

In the majority of experiments the rates of flow in all the vessels approximated the pre-tilting values within 6 to 10 minutes after the animals were returned to the horizontal position. In some instances the flow immediately after the animal was returned was considerably higher than the control value, dropping gradually to the pre-tilting level after 3 to 4 minutes.

In a previous publication (8) we reported that denervation of the carotid sinuses or cutting the vagi, thus eliminating the two aortic nerves, uniformly

diminished the animal's ability to compensate for gravity. Elimination of both pressor receptor mechanisms often resulted in complete absence of compensation and a consequent drop in blood pressure to shock levels from which there was slight recovery. The blood flow changes in the carotid artery in such an experiment are shown in figure 4. Similar changes were observed with respect to the femoral artery and vein and the jugular vein. In the absence of compensatory vasoconstriction the blood pressure and blood flow failed to show

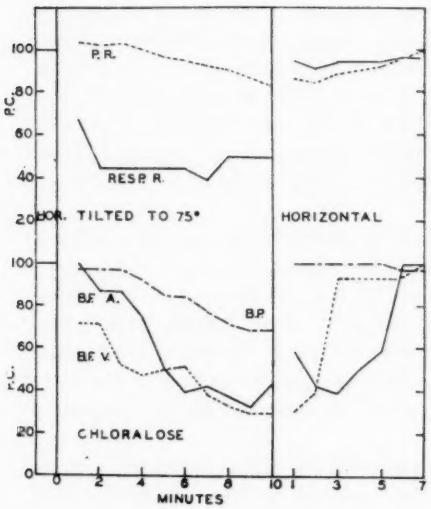

Fig. 3

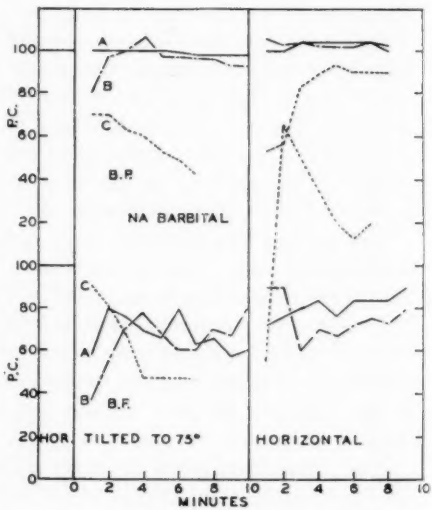

Fig. 4

Fig. 3. Percentage changes in blood flow in the renal artery, *B.F.A.*, and vein, *B.F.V.* Blood pressure, *B.P.*, recorded from carotid artery and corrected for hydrostatic pressure effect. *P.R.* = pulse rate; *Resp. R.* = respiratory rate. Control values: *B.P.* = 150 mm. Hg; *B.F.A.* = 360 cc. per min.; *B.F.V.* = 248 cc. per min.; *P.R.* = 185 per min.; *Resp. R.* = 16 per min.

Fig. 4. Percentage changes in blood pressure, *B.P.*, and blood flow, *B.F.* in the carotid artery. Blood pressure corrected for hydrostatic pressure effect. *A:* intact animal; *B:* after carotid sinus denervation; *C:* after additional cervical section of vagi. Animal returned to horizontal at 7 minutes to prevent complete circulatory collapse. Control values: *A:*—*B.P.* = 165 mm. Hg; *B.F.* = 63 cc. per min.; *B:*—*B.P.* = 175 mm. Hg, *B.F.* = 67 cc. per min.; *C:*—*B.P.* = 205 mm. Hg, *B.F.* = 105 cc. per min.

their usual secondary rise, there being a gradual drop to dangerously low levels which necessitated the return of the animal to the horizontal position to prevent complete respiratory and vasomotor failure and death.

The results obtained in these experiments supplement and extend those previously reported on man in which measurements of circulation times in the upright position indicated a marked retardation of blood flow to and from the lower extremities (1, 9). They provide direct evidence that as a result of the com-

pensatory orthostatic vasoconstriction there is a slowing of the volume flow to the subcardial regions with consequent decrease in renal (7), gastric (10), and intestinal activity (11). This decrease in flow on the arterial side is accompanied by a similar decrease in venous return due to pooling and stagnation in the capillaries and veins which may be particularly marked if the upright position is maintained for more than relatively short intervals of time in the absence of adequate vasomotor and muscular tone (12). Under these conditions the carotid pressure and blood flow may be diminished to such an extent as to fail to meet the demands of the brain for oxygen and a relative anoxemia and consequent circulatory failure may ensue. The more marked changes in the rates of flow in the femoral and renal veins emphasize the possibility that circulatory failure, when it does occur, may be due primarily to weakness on the venous rather than on the arteriolar side of the system.

SUMMARY

Tilting of anesthetized dogs to the upright (75°) position, feet down, resulted in a consistent and marked decrease in the rate of blood flow in the femoral vein and artery and a less pronounced fall in the carotid artery and jugular vein. Changes in renal flow were less consistently observed, but in the majority of experiments were in the same direction.

Acknowledgments. I am grateful to Dr. Julia F. Herrick who was kind enough to send us one of the units used at the Mayo Clinic and who offered innumerable suggestions in regard to the manufacture and use of the units.

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EFFECT OF FASTING AND FEEDING ON THE RESPONSE OF SURVIVING TISSUES TO QUINIDINE

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The multiplicity of factors which modify the behavior of surviving isolated tissues and of enzyme systems necessitates a careful statement of each experimental situation. In this paper the necessity for stating the antecedent nutritional state of the animal, at least in the case of liver slices of the rat, will be demonstrated. The paper concerns the response of the surviving isolated slices of rat kidney and liver, in the presence of adequate antecedent food intake and after enforced fast, to the protoplasmic poison quinidine. This drug contains basic nitrogen in two functional senses and is isomeric with its levo stereomer quinine.

The metabolic actions of quinine on surviving isolated tissues were studied by Druckrey (1935) who reported the initial augmentation by the drug of the utilization of oxygen by "normal" liver slices. He ascribed the initial augmentation to tissue damage. Pennetti (1926), using the Lipschitz (1921) method, which employs dinitrobenzol, found that quinidine inhibits the utilization of oxygen by the surviving liver, kidney and nervous tissue of the dog. The complex nature of the reaction of quinine on the metabolism of various races of yeast has been stated by Rona *et al.* (1923, 1927).

Quinidine and quinine possess pharmacodynamic and therapeutic properties which differ chiefly in degree. Our results indicate that the similarity of action obtains also in the case of the respiration of certain surviving tissues.

METHOD. The Warburg technic (1926) using Dickens and Simer (1931) vessels was employed to measure the oxygen utilization by surviving tissue slices. After the attainment of initial control respiratory level, the drug to be studied, dissolved in normal saline, was introduced from the side-cup into the compartment containing the tissue slices.

Unlike Druckrey's experiments which employed H_2CO_3-$NaHCO_3$ buffered Ringer's solution or inactivated horse serum, these experiments were carried out in a modified Krebs' (1933) buffered saline (pH 7.3) in which the Mg^{++} and Ca^{++} concentrations were halved. This medium contained also 0.2 per cent glucose as substrate. The outer well of the vessels contained KOH to absorb CO_2. The experiments were performed in an atmosphere of oxygen at a temperature of 37° C $\pm$ 0.02. The values for Q_{O_2} were calculated against dry weight of tissue. The various concentrations of the drug used will need to be described in the results for each separate experimental situation.

RESULTS. *Action of quinidine on:* 1. *Slices of rat kidney cortex—fasted and non-fasted.* The results are shown in figure 1 plotted as concentration action

curves. The slices were brought to equilibrium and their oxygen utilization measured for ten minutes. The drug was then introduced. Beginning twenty minutes later the oxygen utilization was measured over the succeeding twenty minute period. Oxygen utilization during this period, *i.e.*, between the 30th and 50th minute after equilibrium (100 to 120 minutes after the death of the animal), was arbitrarily chosen for comparison with the utilization during the control period. In no experiment was there more variation than five minutes in either direction from the specified time for initiating the experimental periods. The ordinates of the curve were calculated by dividing the oxygen utilization during the arbitrarily chosen period of drug action by the oxygen utilization determined at the outset and multiplying by 100 per cent. Since these percentages are necessarily modified by a depression of oxygen utilization arising

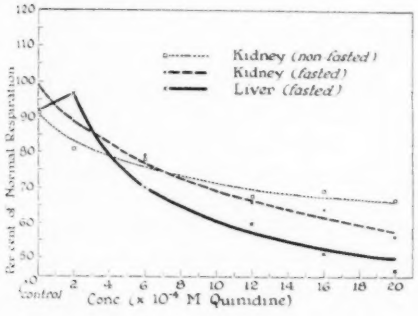

Fig. 1

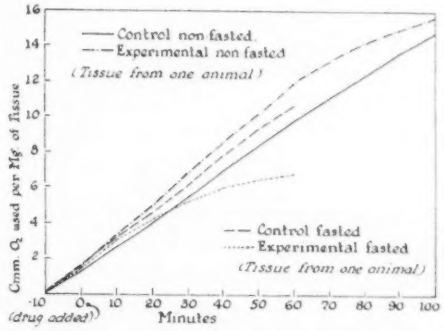

Fig. 2

Fig. 1. Effect of quinidine on the respiration of rat tissue slices, expressed as concentration-action curves. The time selected for the experimental values was from the 20th to the 40th minute after the addition of the drug. The curves for kidney tissue represent an average for five experiments, for liver three experiments.

Fig. 2. Summated oxygen utilization by slices of rat liver (fasted and non-fasted) in the presence of 20×10^{-4} M. quinidine.

from unavoidable tissue deterioration, they are compared in the graph to an ordinate for zero concentration of quinidine (control) in which the percentage response was calculated by comparing the oxygen utilization of untreated slices during the experimental period with that of the control period.

The idealized curve for kidney slices obtained from rats fasted 24 hours represents averages of five experiments. It indicates a depression of about 40 per cent at a concentration of 20×10^{-4} M quinidine. Such a curve for non-fasted kidney slices representing averages of 5 experiments indicates a depression of about 20 per cent for the same concentration. We consider this to be a significant difference.

2. *Liver slices—fasted rats.* The animals were fasted 24 hours before death. The concentration action curves for these slices arrived at by the method used on kidney slices is similar to the curve for kidney slices; a depression of about

40 per cent was produced by the highest concentration of quinidine (fig. 1). This is about the highest concentration which the limited solubility of quinidine permits in the presence of phosphate buffer (M/75, pH 7.3). The curve also indicates, even in the case of fasted animals, a stimulation of respiration with the lowest concentration of quinidine used. It was drawn from the averages of three experiments.

3. *Liver slices—non-fasted rats.* The action of quinidine on liver slices from non-fasted rats is not amenable to representation by concentration-action curves because it is a dual one consisting of augmented and inhibited phases. The curves of figure 2 represent two typical experiments (one fasted and one non-fasted) drawn by plotting the sum of the increments of oxygen utilization per milligram of dry tissue per ten minutes. The control period is repre-

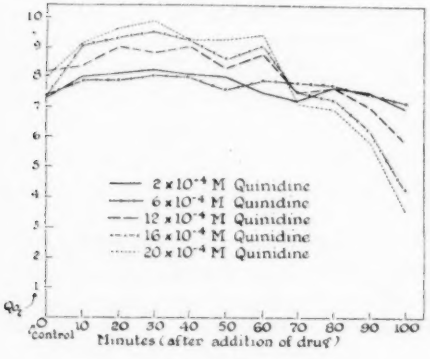
2 x 10⁻⁴ M Quinidine
6 x 10⁻⁴ M Quinidine
12 x 10⁻⁴ M Quinidine
16 x 10⁻⁴ M Quinidine
20 x 10⁻⁴ M Quinidine
control
Minutes (after addition of drug)

Fig. 3

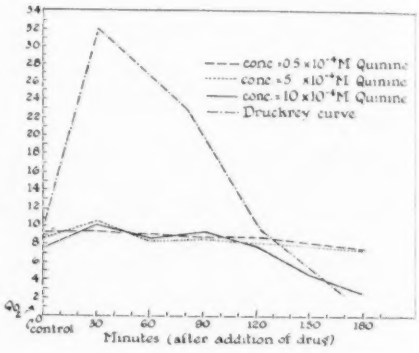

Fig. 4

Fig. 3. Composite time-action curves showing the effect of quinidine on the oxygen utilization of non-fasted rat liver. The curve for each concentration of drug is an average of results from three experiments.

Fig. 4. The effect of quinine on the respiration of non-fasted rat liver slices; comparison with Druckrey's results.

sented by the abscissa from −10 minutes to zero minutes, at which latter time the drug was added to the system. To avoid the confusion of too many lines the results of only the control and highest concentration are graphed for each experiment. Comparison of these curves shows clearly that quinidine initially augments and finally depresses the respiratory metabolism of non-fasted rat liver slices, an effect differing from that with fasting tissue where only depression was observed with this concentration of the drug. The results of these experiments are in excellent accord with those of sixteen other experimental situations. In figure 3 the average time-action curves for three experiments emphasize the dependence of metabolic augmentation upon drug concentration.

Action of quinine on: 1. *Rat liver slices—non-fasted.* The responses of non-fasted liver slices in modified Krebs' solution (pH 7.3) to quinine are plotted in figure 4 as time-action curves in which the ordinates represent Q_{O_2} values and

the abscissas represent time intervals. Results obtained in concentrations from 0.5×10^{-4} M. to 10×10^{-4} M. are compared with those reported by Druckrey (1935) who used carbonic acid-bicarbonate buffer and concentrations of quinine like ours. His procedure included a preliminary treatment of the tissues with 5×10^{-5} M. quinine, which concentration was subsequently augmented during the experiment to reach 5×10^{-4} M. His observation of a 200 per cent initial augmentation followed by final inhibition contrasts sharply with our augmentation of only 20 per cent followed also by depression. The results of our experiments with quinine are similar to our results with like concentrations of quinidine. The limited solubility of quinine prevents the use of concentrations higher than those we report. Our results with quinine on fasted rat liver are like those obtained with quinidine. They are not shown in the graph.

Results with intact animals. We were unable to demonstrate any significant difference between the LD 50 of quinidine sulfate on intact fasted and non-fasted rats. This drug intraperitoneally injected is lethal to 50 per cent of both groups of rats in doses of 105 mgm./kgm. The action on the intact animal is characterized by convulsions terminating in respiratory embarrassment and arrest. Although the convulsive seizures can be controlled by barbiturates, death eventually supervenes. These data were gained through the use of 24 rats.

Discussion. The observations herein reported indicate the importance of inclusion of a statement of the antecedent nutritional state of animals which are used to furnish tissue slices for the study of respiratory metabolism. The presence of glucose substrate in the experimental vessel in no wise minimizes this necessity. The importance of the effects of adequate prior feeding becomes evident when the slices are poisoned by a drug. The difference may be overlooked if the oxygen utilization by unpoisoned slices from fasted animals is compared with that of unpoisoned slices from non-fasted animals. In such situations, providing glucose substrate be present, there seems to be little significant difference between the initial oxygen utilization by fasted and non-fasted tissue slices.

In this study a lapse of two hours after death of the animal has been construed as liminal for satisfactory observation of the gaseous metabolism of surviving tissue slices. If forced by the necessity of comparison with the observations of other investigators, we have, of course, prolonged the time of observation.

It seems that the quinidine induced preliminary augmentation of oxygen utilization by non-fasted liver slices and the contrasting inhibition in the case of fasted slices are related either to the presence of ample glycogen in non-fasted slices or to modification of the detoxicating properties of the fasted slices by the act of fasting or by both. Detoxication might lead to the formation of a derivative of quinidine which agreed in action with the parent drug neither qualitatively nor quantitatively. It is not chemically unthinkable that either quinidine or some detoxication product of it might "compete" with the apoenzyme for iron-porphyrin prosthetic group of cytochrome oxidase.

It is not the purpose of this paper to attempt complete reconciliation of our results in which specificity of action was sought with those which Druckrey reported as essentially non specific. The fact that, in this study, augmentation of respiration of non-fasted liver slices was obtained with quinidine, whereas depression resulted in the case of fasted liver slices, points to the achievement of specific action. The differences between our medium and Druckrey's, particularly with respect to the presence of glucose in ours, may have caused the difference. The divergency cannot be related to a misunderstanding of what Druckrey meant by "normal" animals because liver slices from neither fasted nor non-fasted animals yielded in our experiments such a strikingly large augmentation of respiration as he reported quinine to have produced. Limitations of physical equipment prevented our study of the obviously important glycolytic phenomenon.

The difference between our results and those of Druckrey may also have arisen from the fact that in these experiments PO_4 buffer was used, whereas he relied primarily upon bicarbonate buffer. The concentration of the phosphate in the medium used in these studies (M/60 before the addition of the drug and M/75 after the addition) would appear to be low enough so as not to produce damage to the tissues. Dickens and Simer (1931) have shown that the respiration of a variety of tissues is the same in bicarbonate saline medium as in a M/40 phosphate saline medium. Van Heyningen (1935) showed that at a phosphate concentration of M/100 the reaction of tissues to cyanide is the same as in bicarbonate saline medium. Further, the qualitatively different response of fasted tissue as compared to non-fasted tissue would also preclude the idea of tissue damage as the cause of the observed results. Although any attempt to interpret the results in terms of the concept of phosphate bound energy (Kalckar, 1941; Lipmann, 1941) would seem to be premature, the implication of that concept in interpreting prevention of tissue damage and in detoxication is rationally possible.

CONCLUSION

1. The oxygen utilization by the surviving tissue of rat liver in the presence of glucose substrate and the ions of Krebs' solution is initially augmented by quinidine when the animals have not been fasted; it is depressed when the animals have been fasted.

2. The oxygen utilization by the surviving tissue of rat kidney is depressed by quinidine. The action is qualitatively the same for the tissue of fasted and non-fasted animals.

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THE INFLUENCE OF BENZEDRINE ON WORK-DECREMENT AND PATELLAR REFLEX

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Voluntary muscular fatigue in man has long been studied by the taking of ergograph records of the maximal voluntary contractions of the middle finger of the hand. Using such a method, Mosso (1890) delineated many of the phenomena of voluntary muscular fatigue, and later (1893) observed an increase in voluntary work capacity following the administration of small doses of caffeine. While there have been several later studies of the effects of caffeine upon voluntary muscular work, the effects have been so inconstant and slight that it is not entirely clear today what conditions do determine an increase in work capacity after caffeine administration. However, some of the reported observations do indicate that the central nervous system stimulant effects of caffeine are dominant in the increase in the capacity for voluntary muscular work. The object of the present investigation was to determine the influence of another central nervous stimulant, benzedrine, upon the capacity for voluntary muscular work, and to relate its effects on work capacity to another central nervous system stimulation phenomenon that could be coincidentally observed. In this connection, observations of patellar reflex activity were made during the rest intervals between observations of the work capacity of the middle finger.

EXPERIMENTAL STUDIES. A Mosso-type ergograph, in which a weight was lifted by the middle finger and a kymograph record made of the movement of the weight, was used. The index and ring fingers were inserted into two rigidly anchored metal tubes, and the forearm was strapped in place on the base of the apparatus. The weight was kept constant during any given series of experiments, and the contraction effort was made in time with a metronome operating at 36 beats per minute until no further effective movement of the weight occurred. The subject was instructed to make a maximal contraction effort, involving flexion of all the joints, with each beat of the metronome, as Reid (1928) found that, outside of some modification in the character of the fatigue curve profile, little difference was found between the tracings made with the first or first and second interphalangeal joints in restraint and those in which free movement of all phalanges was permitted.

For study of patellar-reflex activity, a chair was designed in which the seat sloped to the rear to produce a maximum tension on the patellar tendon, and the back made a wide angle with the seat to afford a semi-reclining position to facilitate complete relaxation. An electromagnetic hammer positionally adjustable to the individual was used, and its frequency and intensity of stroke were con-

trolled by a condenser-discharge timer. The amplitude of the movements of the foot was recorded with a kymograph or a pen-writing polygraph.

Observations were made on six normally healthy adult males ranging from 22 to 38 years in age, and each series of observations on an individual was begun in the morning, one to two hours following a light breakfast, and was continued without further food intake until completed. Initially, the subject relaxed in the reflex chair until a steady state appeared to have been reached, then the initial recording of patellar-reflex activity was made. Following this, the subject walked a few paces to the ergograph and made a work capacity record, then returned to sit relaxed in the reflex chair until the time of the next observation.

In the initial studies, the ergograph was loaded with 2.7 kgm. and periods of 30 minutes between observations were used with all six subjects. One or two series of normal observations without any drug administration were made. Then a series of observations was made in which, immediately after the initial observations of patellar-reflex activity and work capacity, there was taken orally 10, 20 or 40 mgm. of benzedrine sulfate with a small amount of water. The initial observations thus furnished a basis for comparison of work capacity and patellar-reflex activity in the series on any one day, so that the work decrement or changes in reflex activity could be relatively valued with repeated observations.

Figure 1 shows graphs of this type of comparative study, parts A and B showing the decrement in work capacity of G. A. occurring in the repeated tests made during control periods of two hours on different days, during which the patellar-reflex activity varied but little. Parts C and D of figure 1 show the graphs of work capacity and patellar-reflex activity when a 20 mgm. dosage of benzedrine sulfate was taken. While the initial decrement in work capacity shown in C was much like that in A or B, the work capacity was increased at the 60-minute observation as compared with that at 30 minutes; and at the 90, 120 and 150-minute observations the work capacity was even greater than the initial work capacity. At these same times, the patellar-reflex activity was very notably increased, as shown in part D.

The observations made with the six subjects are summarized in table 1 with valuations of the work capacity and patellar-reflex activity made relative to the initial observations. The work values were compared by determining the areas of the kymograph tracings, since the kymograph speed, contraction frequency and load were constant throughout any experimental series. In the tabulation the initial work values were taken as 100, and succeeding work values expressed in per cents of this value. The initial reflex activity was given a rating of one plus and succeeding activities rated in comparison by using one or more plus values. As shown by these data, following a dosage of 10 mgm. of benzedrine sulfate there was an increase in work capacity, evident about 30 minutes later, though sometimes requiring 60 to 90 minutes, and even longer, before the effect was clearly evident. The usual effect was not to increase the work capacity above the initial level, but was simply an action against the decrement occurring in successive observations. The patellar-reflex activity was quite regularly observed to be increased at times of increase in work capacity or at times when the

work capacity was greater by comparison with the normal decrement of successive observations in the control series. Usually the higher dosages gave more definite effects with regard to increases in work capacity and patellar-reflex activity, but this was not always evident, particularly as shown by R. I., in whom 20 mgm. appeared to have less effect than 10 mgm. on work capacity.

In the course of doing the preceding experiments, it became apparent that, although all the individuals were leading regular and temperate lives and the details of observation were held as constant as possible, there was on occasion an unpredictable 40–70 per cent variance in the initial work capacity of these individuals. Correspondingly, the work decrement found on any one day would vary considerably as compared with that of another day, and it soon became apparent that some modification of technic was desirable to make clearer the effect of benzedrine sulfate upon work capacity or its decrement as studied.

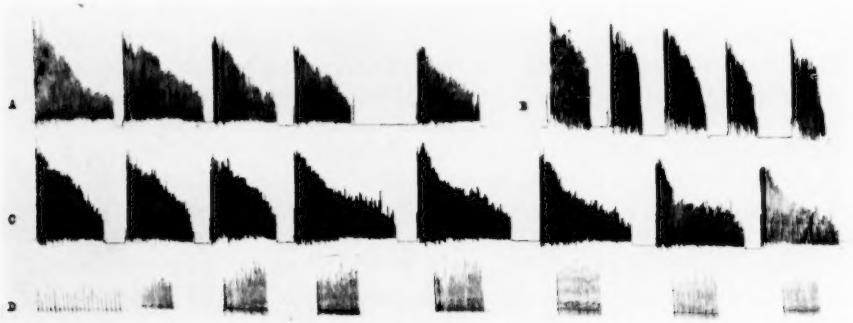

Fig. 1. A and B. Total finger work capacity on different days at successive trials every 15 minutes. C. Total finger work, and D, patellar reflex activity on another day at successive trials every 15 minutes following an initial taking of 15 mgm. benzedrine sulfate orally.

To obviate these difficulties, so far as they interfered with the clear demonstration of the effect of benzedrine sulfate upon work capacity, a different routine of observation of its effects was developed. This consisted of determining for each individual the effective load which, when observations were repeated every 15 minutes, would give a normal work decrement series in which the work capacity became negligible within a two-hour period. Then, at two hours, when the work capacity was at this level, administration of the drug and continuation of the work capacity trials (without interruption) demonstrated clearly the effect on work capacity, and permitted some estimation of the duration of that effect. Loadings of from 2.7 to 5.0 kgm. were required for this type of study with the same persons previously studied. Figure 2 shows the graphs of the work done with such an experimental procedure by G. F., before and after 10, 20 and 40 mgm. of benzedrine sulfate as used in the different experiments. It is to be noted that marked effects were present for three hours after administration of but 10

TABLE 1

Finger work capacity and patellar-reflex activity

	BENZEDRINE SULFATE	TIME OF OBSERVATIONS IN MINUTES								
		Initial	30	60	90	120	150	180	210	240
	mgm.									
G. A.	0	100	86	92	76	78	–	–	–	–
	0	100 +	105 +	99 +	76 +	85 +	– –	– –	– –	– –
	20	100 +	87 ++	92 +++	122 +++	128 ++++	103 ++++	94 +++	85 +++	– –
	40	100 +	132 ++	145 +++	107 +++	105 +++	109 +++	98 ++	96 ++	– –
G. F.	10	100 +	79 +	46 +	34 +	32 ++	50 +++	54 ++	– –	– –
	20	100 +	70 +	60 +	49 ++	58 +++	29 ++	30 ++	– –	– –
	40	100 +	92 +	58 ++	71 ++	87 +++	62 +++	58 ++	50 ++	53 ++
	0	100	86	80	64	70	68	48	–	–
	0	100	108	102	100	85	88	75	–	–
P. S.	0	100	88	62	51	38	40	42	–	–
	10	100 +	122 ++	105 +++	14 ++++	9 ++++	44 +++	34 ++	– –	– –
E. R.	0	100	110	102	75	60	55	64	–	–
	10	100 +	130 +	133 +++	92 +++	89 +++	76 ++	66 ++	– –	– –
	20	100 +	129 +	138 ++	137 ++	111 +++	121 ++	80 ++	– –	– –
R. I.	0	100	61	13	5	3	5	4	–	–
	10	100 +	95 +	85 ++	49 ++	38 ++	47 ++	45 ++	– –	– –
	20	100 +	57 +	30 ++	9 +++	5 ++	2 ++	1 +	– –	– –
C. B.	0	100	74	55	41	36	43	36	–	–
	10	100 +	130 ++	121 +++	98 ++	100 +++	90 ++	94 +	– –	– –
	20	100 +	100 +	58 +	66 +	137 ++	138 +	127 +	– –	– –

Benzedrine sulfate taken orally after initial observation.

Work capacity in per cent of initial value—reflex activity in + of initial value.

mgm., and the 20 mgm. experiment was continued to five hours with no great further decrease in work capacity at this time as compared with the capacity shown at three hours. Similar experiments were also carried out with four of the other five subjects worked with previously, and with each individual similar striking recoveries of work capacity were demonstrated following dosages of 10 mgm. or more of benzedrine sulfate. These effects were evident in all cases within 60 minutes after administration orally. Increases in patellar-reflex activity were also evident at times of increased work capacity, and there was a fair time correspondence in the degrees of these two effects.

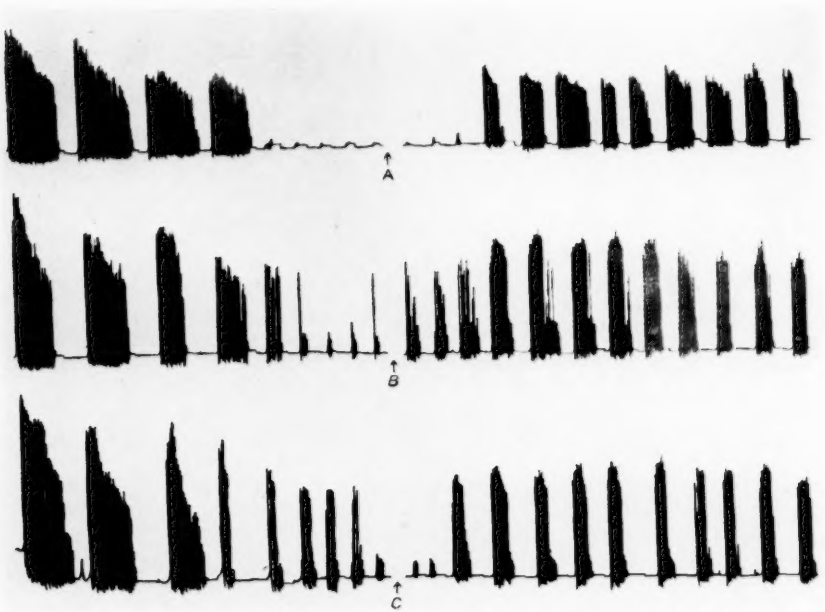

Fig. 2. Total finger work capacity on different days with benzedrine sulfate after initial two hours. In first series, at A, 10 mgm. administered orally after 8th test trial and the 9th test trial made on schedule 15 minutes later with successive trials continued for 3 hours after. In second series, at B, 20 mgm. were administered and in third series, at C, 40 mgm.

At the beginning of the present work it had been decided to use caffeine as a comparison drug for the fatigue studies. Preliminary experiments on the authors with dosages of 100, 200 or 400 mgm. of caffeine taken orally failed to show any definite results, the effects, if any, being within the normal daily variance. With the technic of first carrying out observations to a negligible work capacity level, it was considered possible to compare directly the effects of caffeine and benzedrine on fatigue. These attempts were carried out by orally administering, in different experiments, 100, 200 or 400 mgm. of caffeine (200, 400 or 800 mgm. of caffeine citrate) after the initial two-hour work decrement period. Then, after a

second two-hour or longer observation period of caffeine effects, a dose of 10 mgm. of benzedrine sulfate was orally administered and its effects on work capacity noted during a subsequent two-hour period. The results obtained were, however, rather equivocal and inconstant due to the influence of variables inherent in the training process.

The first series of experiments of the "exhaustion" type were performed using caffeine alone. In all tests included in this series the right hand was used and the weights were the same as those used for each subject in the previously described benzedrine experiments. The work capacity of G. A. was undiminished at the end of the 2-hour initial period in all tests of this series, and the various dosages of caffeine were incapable of increasing work output above normal. In the first experiment of this series with G. F. a low work-capacity level was approached at the end of 2 hours, and subsequent administration of 200 mgm. of caffeine citrate produced slight increase in subsequent work performance. In the following experiments, involving 400 and 800 mgm. of caffeine citrate, the initial 2-hour period did not result in work-capacities low enough to permit satisfactory basis for judgment concerning the influence of caffeine on the work curves following its administration at the conclusion of the initial 2-hour control period.

Failure to produce sufficient decrease in work capacity in the initial control period in the foregoing first series of experiments prompted the authors to change the hand undergoing fatigue, and to increase the loads used. In a preliminary experiment with G. F. where the working hand was changed from the right to the left, but the load left unaltered, no considerable decrement in work capacity occurred during the initial control period, although this was the first time that he had used that hand in an ergograph experiment. Under these conditions, the administration of 200 mgm. of caffeine citrate resulted in no change in the work curves during the continuation of the observational period.

Repetition of the experiment with an increased load produced complete exhaustion in less than 2 hours, which was not relieved by 200 mgm. of caffeine citrate in the two hours after its administration, but which subsequently responded to 10 mgm. of benzedrine sulfate with a marked increase in work capacity within 30 minutes of its administration. In the following experiment with the increased load, there also was complete exhaustion during the initial test period, and the administration of 400 mgm. of caffeine citrate restored the work capacity for 4½ hours before exhaustion again set in, and then 10 mgm. of benzedrine sulfate caused a marked restoration of work capacity for the additional 3½ hour period during which observations were continued. In another experiment, after the initial control exhaustion period, 800 mgm. caffeine citrate produced an increase in work-capacity for 7½ hours, but the side-effects of irritability and unsteadiness were rather extreme. Attempts to confirm these observations with regard to caffeine effects on work capacity were unsuccessful due to the fact that it was impossible to find a suitable load to produce consistently the initial degree of fatigue required. The final weight tried with the left hand, and which failed to produce the initial fatigue, was three times as great as the one which had been used with the right hand in the initial experiments cited in this paper.

In similar experiments with G. A., after changing to the left hand instead of the right and increasing the load, neither the 200 nor the 400 mgm. doses of caffeine citrate showed demonstrable effect, although the subsequent 10 mgm. dose of benzedrine sulfate showed quite a definite work-capacity effect in the continuation of the experiments. In the experiment involving the 800 mgm. dose of caffeine citrate, the work capacity did not decrease suitably during the initial control period, and in consequence no definite caffeine or subsequent benzedrine effects were observed. Increasing the load in a following experiment, under the same dosage regime, also failed to produce a definite result.

The amplitude of the patellar reflex was materially unchanged in all of the studies on both G. F. and G. A. following caffeine administration, though considerable jitteriness and unsteadiness were observed by G. F. following the higher doses of caffeine citrate.

Because of training and rapid adaptational factors, that may permeate any set of experiments of this type, very definite conclusions cannot be drawn as to the effects of caffeine upon work decrement. However, it is evident that, when therapeutically useful doses of both compounds are given in a single experiment, benzedrine sulfate is more effective than caffeine citrate in producing a return toward normal of voluntary muscular activity after "exhaustion".

The effects of training were well illustrated during the first part of the studies here reported in that, during the consecutive experimental trials on different days, there was fairly constant improvement with time, and this might exceed the apparent effects of administration of benzedrine sulfate or caffeine citrate on any one day. In the later experiments of the exhaustion type, this phenomenon was also noted when the subject failed to become exhausted with a given weight which had been adequate to bring his work capacity to a low value in the same time during the preceding experiments of a series.

The transfer of training from one hand to the other was also noted in these experiments, which may be considered to support the idea that the work capacity is limited by a central nervous system mechanism. In the case of G. F., at the beginning of the present ergograph studies which ran over a period of one and a half years, he was adequately exhausted within the initial two-hour period with a weight of 2.7 kgm. when using his right hand. As the experiments progressed, the loading had to be increased to 4.2 kgm. After adaptation to this load, the left hand was employed with the same weight, with resultant rapid adaptation, and at the conclusion of these experiments of this paper 8.5 kgm. was an insufficient load to produce decreases in work capacity levels during the initial control period.

Discussion. The mechanism of action of drugs in increasing the capacity to do voluntary muscular work has chiefly been considered in the past with respect to the effects of caffeine. Mosso (1893) was the first to observe an increase in ergographic work following the administration of caffeine, but this phenomenon was much more extensively studied by Hoch and Kraepelin (1896) and by Oseretzkowsky and Kraepelin (1901). These latter authors reported that caffeine acted to increase the extent of each contraction, but not the number of contrac-

tions, before fatigue. From the studies of Kraepelin and his co-workers it was considered to be established that the number of contractions was primarily related to the state of the nervous system, and their extent related to the state of the muscle. The conclusion was drawn that caffeine acts directly upon the muscle in producing its effect of increasing muscle work. Of the several studies on ergographic work effects of caffeine in man since carried out, the work of Rivers and Webber (1907) is of greatest interest in connection with proposed mechanisms of action. They found that in the two subjects extensively studied the effect on the number and extent of contractions was quite different, in that the one (W.) the effect was predominantly upon the extent of contractions, and in the other (R.) the effect was predominantly upon number, though both effects could be seen in either individual under particular conditions. This demonstrated that caffeine exhibited a double action in its effect on capacity for muscular work, and it was pointed out that it was well-known that caffeine acts on the isolated neuro-muscular mechanism and on the capacity for mental work. It thus appears that there is no definite conclusion yet to be drawn as to the exact site of action of caffeine in producing its effect on voluntary work capacity in man, though it often does exhibit a double action, and this doubleness of action may be related to dual mechanisms of action.

More recently the mechanism of voluntary muscular fatigue in man has been further investigated by Reid (1928) who was able to establish that any failure of the peripheral neuro-muscular mechanism is not an important factor in voluntary fatigue. Only with very rapid contraction rates under well-loaded conditions (120–160 per min., with 2 kgm. or more) may there be a peripheral effect overlapping the central cause of voluntary fatigue, while with a slow series (12 to about 80 per min.) there was little evidence for reduction of muscle contractile power. It is of interest, in connection with the present studies on benzedrine, that the blood supply to the muscle under conditions of extreme limitation can determine the voluntary muscular fatigue from serial contractions and that the earlier central failure is attributable to the influence of afferent inhibitory impulses from the working muscle.

In the present studies with benzedrine, the effects upon work production were not clearly either an increase in extent of contraction or an increase in duration of contractions, but rather an effect on both, though on occasion one or the other effect was dominant. In general, the effects upon duration of contraction were the more notable under the earlier experimental conditions, where benzedrine was taken immediately after the initial observations. When the benzedrine was taken after complete fatigue had set in, as in the later series of experiments, the effect on extent of contraction was the notable effect, the duration of such effects being quite constant over a considerable period of time. Benzedrine, like caffeine, thus appears to have a double aspect in its action upon voluntary muscular fatigue, but it is not clear that this is to be related to a duality of mechanism of action.

The fairly close correspondence in the present experiments of the increases in patellar-reflex activity with increases in muscular work capacity would indicate

that these are similar actions of benzedrine. While benzedrine exerts other central nervous system stimulant effects, those that are best known are usually of appreciably longer duration from the same dosages. The anti-sleep and mental stimulant activities resulting from 10, and particularly from 20 or 40 mgm. of benzedrine sulfate persisted through the succeeding night with most of the subjects of the present studies, though definite voluntary work or patellar-reflex effects usually passed through a maximum in from two to five hours after administration. While the higher doses (20 and 40 mgm.) of benzedrine sulfate used in some of the present experiments do exert detectable systemic circulatory effects, it is doubtful that such effects directly affected work capacity by changes in the muscle circulation, since the muscle circulation does not normally appear to be a limiting factor in voluntary muscular fatigue. Whether or not circulatory changes in the region of the central synapses involved exert a limiting effect cannot be speculated upon to advantage at present.

SUMMARY

1. Doses of 10, 20 or 40 mgm. of benzedrine sulfate inhibit the production of voluntary muscular fatigue.

2. Doses of 10, 20 or 40 mgm. of benzedrine sulfate may abolish essentially complete voluntary muscular fatigue maintained by repeated but static work trials.

3. The effect may be exerted with respect to increase in extent of each contraction, or in number of contractions, or both.

4. The effect is related fairly well in degree and time with the patellar-reflex activity effects of benzedrine sulfate.

5. The effect is probably primarily related to an action directly on the central nervous system.

6. The effect with benzedrine is more marked than can be observed with ten times as great a dosage of caffeine under the same conditions.

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OBSERVATIONS ON HEMORRHAGIC HYPOTENSION AND HEMORRHAGIC SHOCK[1]

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During the past year we have restudied the dynamics of the circulation during and after regulated hemorrhage and after reinfusion of the blood withdrawn in the hope that an objective analysis of results would crystallize answers to a number of moot questions. Our primary interests consisted in determining whether hemorrhage *per se* can induce shock and, if so, the conditions for its production, the possibility of standardizing the procedure, the criteria for its recognition and the nature of the eventual failure. Our study strongly suggests that some accepted notions as to the mechanisms concerned in the progression and irreversibility of the circulatory state we call shock may need to be amended.

ANESTHESIA. Anesthesia is essential for such dynamic studies. Ether and other volatile anesthetics have not proved satisfactory, because their uneven concentration in the blood and respiratory passages causes humoral and reflex actions which complicate and confuse the circulatory reactions due to hemorrhage alone.

Most of our dogs were given a preliminary subcutaneous injection of morphine sulfate (ca, 3 mgm./kilo) and just enough sodium barbital intravenously (ca, 175–200 mgm./kilo) to complete the anesthesia. In a few experiments sodium amytal[2] (ca, 40 mgm./kilo) was used. The reasons for our choice of barbiturates have been discussed recently (1). However, as further checks, five experiments were carried out under chloralosane anesthesia (ca, 7.5 mgm./kilo). We have discovered no essential differences in the dynamic reactions aside from the fact that the heart rate is generally—not always—slower when chloralosane is used, and, consequently, the primary cardiac acceleration during hemorrhage is greater.

METHODS. Our standard technical procedure involved optical registration of central arterial pressures from the innominate artery and of central venous pressures from the superior vena cava by high frequency manometers of the Gregg type. In addition, intrathoracic pressure was recorded by a segment capsule which was connected to a trocar inserted through the chest wall into the mediastinal space. All recorders were calibrated repeatedly with respect to individual base lines.

In some experiments we made hematocrit readings or noted the appearance of an exteriorized loop of gut. In a number of experiments, one or both vagus

[1] This investigation was supported by a grant from the Commonwealth Fund.

[2] We are indebted to Eli Lilly Co., Indianapolis, for the sodium amytal.

nerves were divided early during the experiment for testing of neuropressor reactions. Operative procedures were always kept at a minimum.

PROCEDURE. Thirty-five experiments were satisfactory in all respects, but corroborative information was derived from many others. Blood was generally withdrawn from the femoral artery into a solution of heparin, but in a few cases the whole animal was heparinized.[3]

The plan of our experimentation involved reduction of pressures to low levels by bleeding, maintenance of such levels, as nearly as possible, for various intervals, and eventually reinfusion of the whole quantity of blood withdrawn. To achieve a posthemorrhagic hypotension, various expedients previously suggested by others were tried. In general, three methods are useful for particular phases of such studies:

1. *Stepwise bleeding*, *i.e.*, successive withdrawals of blood volumes, each equal to about one per cent body weight, a reasonable time for recovery being allowed between bleedings. The method is useful in assessing the compensatory mechanisms called into action, but proved less suitable for our purpose because it is difficult to judge the last critical volume that may be withdrawn without jeopardizing the life of the animal.

2. *Continuous slow hemorrhages* (ca, 3–10 cc. per min.) over a period of hours until pressures reach the low level desired. This probably most nearly resembles natural methods for depletion of blood volume, but has the disadvantage that experiments are unduly prolonged and that the intensity of the generalized ischemia is difficult to evaluate on a pressure-time basis.

3. Combination of an initial large, rapid hemorrhage and, after a period of recovery, continuous slow hemorrhages until the desired level of pressure is reached; but bleeding a little more whenever pressure rises above the desired level or reinfusing small amounts when danger threatens. This method has several practical advantages: 1. The duration of an experiment can be materially shortened and, as far as we have been able to judge, without modifying the course of events. 2. It is less difficult to hold pressures at a chosen level for a definite time, hence the degree of generalized ischemia can be estimated more accurately.

RESULTS. Results from employment of the last two methods of regulated bleeding may first be briefly analyzed. Segments A to C of figure 1 illustrate the effects of a large and rapid hemorrhage (2 per cent body weight in 2 min.) on the arterial pressure pulses of a dog under sodium amytal anesthesia. Segment D illustrates the recovery attained four minutes later. As a result of the hemorrhage, the heart rate increased almost at once from 160 to 220 per minute, and pressures fell from 153/118 in A to 106/82 in C, recovering to 132/111 mm. Hg in D. Such pressure readings, however, conceal significant differences in the cyclic changes of pressure indicated by the pressure pulses. The central pressure contour in segment A is characteristically normal and need not be described again. The pulse immediately following a large sudden hemorrhage

[3] We are indebted to Dr. L. Klein of Hoffman La Roche, Inc., Nutley, N. J., for the generous supply of Liquaemin which proved highly satisfactory for intravenous injections.

(segment C) displays a large initial spike and a second broader peak during midsystole. Sometimes the initial spike is much larger than in this illustration. The question arises, where systolic pressure should be measured. Since the accentuated spike is due to a momentary overshooting of pressure in the lax arterial system and the midsystolic summit accords with the pressure maximum in the left ventricle, the latter is the more reasonable index. It may be added parenthetically, however, that such spikes may correspond more nearly to auscultatory readings of systolic pressure. If so, sphygmomanometric readings taken clinically would give a false impression of the true systolic pressure. Following a deep incisura and an accentuated after-vibration, the diastolic pressure is nearly horizontal, indicating that no significant "run-off" from the aorta occurs during diastole. This means that tissues receive essentially a periodic systolic flow of blood.

Recovery in the pulse form as well as pressure levels occurs quickly as the aorta is again filled with blood. This is not due to further increase in heart rate; indeed, the rate is slightly slower in segment D than in C (172/min. *vs.* 220/min.). Improved systolic discharge, reduction in capacity of the aorta or increased peripheral resistance may all be concerned. Such arterial records do not permit conclusions as to which, if any, is prepotent. However, we can say that vasoconstriction is far from maximal at this time, for clamping of the carotid arteries caused as great an increase in pressure as it did normally. The right vagus was cut and stimulation of the central end likewise produced a great increase in blood pressure and changes in contour characteristic of an increase in peripheral resistance.

Two other features frequently noted during or after recovery from large hemorrhages are of significance, *viz.*, the development of pronounced Traube-Hering waves and cardiac alternans. The former often caused extreme fluctuations of pressure, but these usually disappeared in 20 to 30 minutes. Ventricular alternans, though less frequent, was by no means uncommon. It is unquestionably brought out by the high heart rates, but often it did not occur until the initial and maximal acceleration had moderated considerably. This suggests that impairment of coronary flow may be quite as important, and that the myocardium does not escape effects of large hemorrhages. Such alternans also was not permanent; presumably some adaptive mechanisms came into action despite a further decline of arterial pressures.

Segment E shows the status after recovery from the initial hemorrhage after a few tests of the animal's pressor responses had been made. These were found to remain normal. The effects of the second progressive and slow hemorrhage are illustrated in segments F to J. As the pressures again decline, the pulse contours do not alter significantly. Details are given in the legends. In segment H, pressures are lower than in segment C, but the spiked feature and flat diastolic characteristic are not shown. As bleeding continued and diastolic pressures became still lower (segments I, J), these features reappeared. The longer persistence of pressure pulses of good form during a slow hemorrhage suggests that the compensatory mechanisms which act *after* a large hemorrhage to

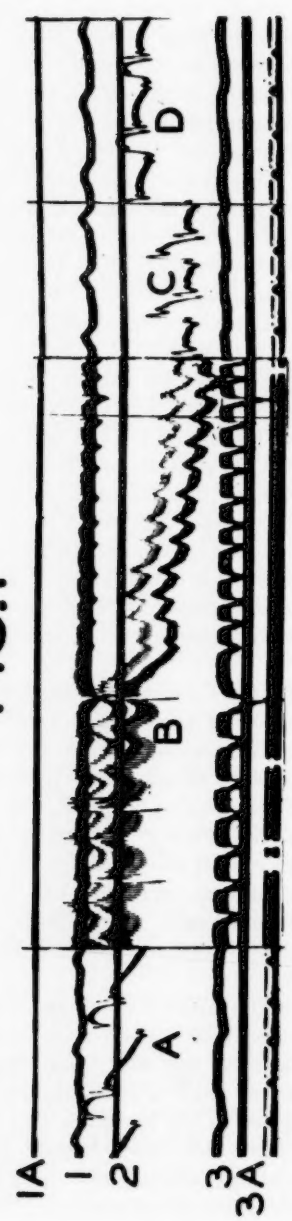

FIG. 1

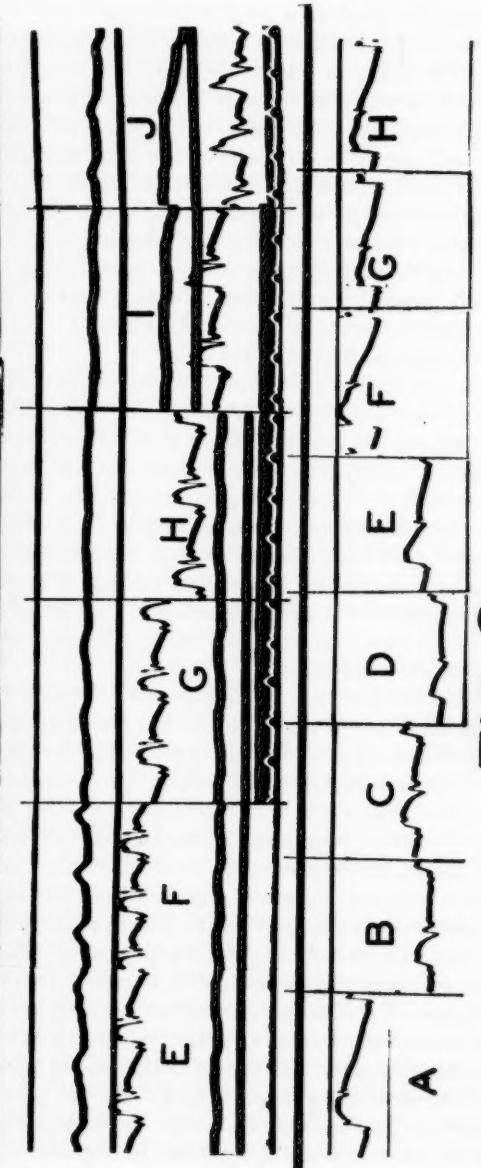

FIG. 3

abolish these features (C, D) operate progressively *during* a slower hemorrhage to prevent their appearance.

This interpretation is supported by experiments in which blood pressures were reduced by slow hemorrhages from the start. Thus segments A to F of figure 2 show the effects of a fairly continuous bleeding at an average rate of 3 cc./min. over a period of 2 hours, 28 minutes. With the decline of blood pressure and diminution of the pulse pressures no tendency existed either to spiking or complete flattening of the diastolic limb until segment F. At this time 435 cc., or a quantity of blood equal to 4 per cent body weight, had been withdrawn.

This curve illustrates other aspects of our problem. Shortly after the record of segment F had been taken bleeding was discontinued owing to the fact that the bulb of our projection lantern had burned out. This altered the relative pressure relations in our curves and recalibration was necessary for the other curves reproduced in the second and third rows. Segment G shows the recovery attained 45 minutes later. Since arterial pressures were still 98/72, an additional 30 cc. was slowly withdrawn. The effects of this critical bleeding are shown in segments H and I. In addition to the marked spikes of the pressure curves the heart slowed significantly. This was of sinus origin as shown by the venous pressure curve above. It persisted when both vagus nerves were cut and therefore appeared to be of cardiac origin. As a result of many observations, we are convinced that when the initial acceleration of the heart after large or continuous slow hemorrhages is followed by significant slowing this constitutes a danger signal. As in this animal, respiration soon ceases and the heart becomes extremely slow, as shown in segment J, and the animal dies unless restorative measures are promptly instituted.

Fig. 1. Central venous pressure (1), central arterial pressure (2), intrathoracic pressure (3). Base lines for same (1A), (2A), (3A). Time 0.2 sec. Description in text

	Time	H.R.	B.P.		Time	H.R.	B.P.
A	11:53	132	153/118	G	12:38	172	111/98
C	11:56	220	106/82	H	12:45	150	90/72
D	11:59	172	132/111	I	1:05	150	77/59
E	12:32	160	132/112	J	1:24	200	56/38
F	12:34	200	128/111				

Fig. 3. Central arterial pressures during and after hemorrhage and recovery following infusion.

	Time	H.R.	B.P.		Time	H.R.	B.P.
A	10:12	93	132/103	F	3:21	120	140/100
B	10:40	144	60/45		(after completion of infusion)		
	(after hem. 3% B.W.)						
C	11:22	147	68/50	G	4:57	120	128/105
	(after hem. 4% B.W.)						(recovery)
D	2:37	132	37/25	H	5:10	120	122/102
	(after hem. 5% B.W.)						(recovery)
E	2:52	123	67/48				
			(small inf.)				

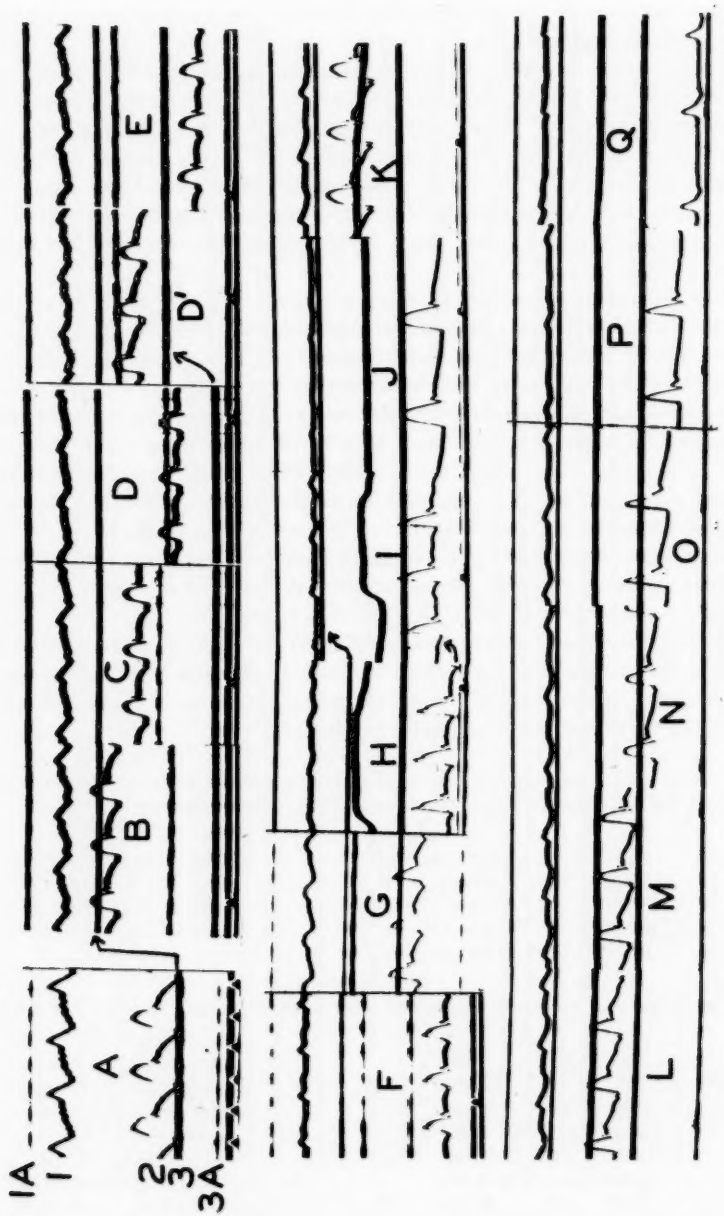

FIG. 2

However, if artificial respiration is given at once and blood is quickly reinfused, the arterial pressures and pulse forms may be restored to normal when all the withdrawn blood has been reinjected. If the pressure pulses of such animals are observed further, however, it is found that some of them maintain arterial pressures and normal pulse forms for three to four hours, whereas others rapidly deteriorate and are dead within 30 minutes to 2 hours. The animals of the first group which nearly died from hemorrhage were rescued through reinfusion of blood; the animals of the second group displaying identical cardiac and arterial pressure changes were only temporarily improved and, we believe, died of hemorrhagic shock. In other words, pronounced posthemorrhagic hypotension may exist for long periods and animals may even die from cardiac or respiratory failure without existence of a circulatory failure that cannot be reversed by substantial injections of blood or plasma. It is important to distinguish death from such *post-hemorrhagic hypotension* from that due to *hemorrhagic shock*, from which dogs cannot be rescued by transfusion.

An example of a favorable response to transfusion is shown in segments D–H of figure 3, the details of which are given in the legends. At the time when segment D was recorded the animal had been bled 5 per cent body weight and had been kept at a mean arterial pressure level of 50 mm. Hg or less over $3\frac{1}{2}$ hours. After a small sustaining infusion of 60 cc. (segment E), the circulation and respiration were maintained for another 35 minutes. Reinfusion of all the withdrawn blood at a critical stage led to restoration of a normal level and form of arterial pressure which was still maintained three hours after infusion (segment H).

An example of an unfavorable ending is illustrated by the remaining curves of figure 2. After reinfusion of the entire volume of withdrawn blood over a period of 46 minutes, the degree of recovery shown in segment K was achieved. Pulse contour and pressure relations were essentially normal; and, moreover, showed no marked changes during the next 30 minutes. However, this state of recovery did not persist. As illustrated by the lower row of segments, pressure progressively declined, the pulse forms deteriorated much as before reinfusion. Toward the end, systolic ejection shortened, the heart again slowed and the animal died within an hour. We again note that estimations of systolic and diastolic pressures alone do not express the degree of circulatory failure, as well as do the pressure pulses.

Fig. 2. Central venous pressure (1); central arterial pressure (2); respiration and arterial base line (3). Base lines for venous pressure (1A), for respiration (3A). Time 0.02 sec. Description in text. Arrows indicate base line shifts between segments.

	Time	H.R.	B.P.		Time	H.R.	B.P.
A	10:44	172	180/137	J	3:37	105	67/25
D	12:12	180	80/62	K	4:26	160	135/92
D^1	12:35	180	125/100	L	5:19	165	108/80
E	12:48	188	60/44	M	5:30	165	104/70
F	2:02	210	61/35	N	5:40	136	78/50
G	2:49	198	98/72	O	5:44	130	75/35
H	3:01	198	80/42	P	5:45	113	63/30
I	3:27	188	70/32	Q	5:46	105	27/13

The records of figure 4 illustrate a number of additional dynamic facts in a dog under chloralosane anesthesia. Segment A shows the marked sinus arrhythmia which resembles that of unanesthetized dogs at rest. It illustrates admirably the impossibility of assigning definite numerical values to heart rates or to systolic and diastolic pressures. When such control values are reported by investigators who used unanesthetized animals these must either be very approximate or their animals were not in a basal, resting state when experiments were begun.

The effects of an initial large hemorrhage (2 per cent body weight) and recovery therefrom are illustrated in segments B and C. In the latter, slowing and some sinus arrhythmia redeveloped. Such slowing while pressures are still good must not be confused with the dangerous slowing when pressures are extremely low. The consecutive effects of a subsequent slow continuous hemorrhage are indicated in a few selected segments, D, E, F, G, H. Details are given in the legends. Notice that beginning with segment G, both the arterial pressure pulse and base line were moved up. The development of the typical spiked curves and flat diastolic limbs need not be redescribed. It is obvious that development of these dynamic changes was not affected by a change of anesthetic.

At H, the animal had been at a mean pressure of about 40 mm. approximately 30 minutes. Although the animal was breathing well and the heart had not started to slow, a sustaining infusion of 40 cc. blood was deemed advisable because pressures had been at 48/25 mm. Hg for some time. At this time, central vagus stimulation caused a definite and large increase of pressure and intensified the peaked character of the pressure pulse (not illustrated). The animal maintained its respiration with a gradual decline of pressures during the next two hours. At the end of that time, marked slowing of the respiration and of the heart, shown in segment J, supervened. Reinfusion was therefore started promptly, first at a rapid and then at a slower rate. The end result is shown in segment K. Pressure relations were restored to normal ranges (135/95) but only a fair restoration in the form of the pressure pulse was achieved. Segment L shows a curve recorded 10 minutes later. Some lowering of pressures, cardiac acceleration and a further abbreviation of the systolic ejection period were conspicuous changes. Pressures were maintained fairly well for another 30 minutes, then progressive cardiac slowing supervened (segment M) and the animal died. Autopsy revealed no coronary embolism or pulmonary edema.

As a result of many similar observations we feel justified in the conclusions that a state of irreversible circulatory failure is demonstrated when, after a period of marked hypotension induced by hemorrhage, arterial pressures and pulse forms fail to be restored more than temporarily by reinjection of the blood previously withdrawn. In other words, hemorrhagic shock exists. Obviously, also, the ultimate failure occurs despite an adequate circulatory volume in the cardiovascular system. The factor which fails resides in the cardiovascular system, not in the blood volume available.

Comparison of experiments such as are illustrated in figures 2 and 4 suggests a possible difference in the mode of failure. In the former, circulatory failure after reinfusion occurs more gradually, venous pressures rise and the slowing of the heart is a terminal feature. In the latter and less numerous instances, pressures are maintained for an hour or more, than quite suddenly the heart slows, arterial pressures decline, and respiration ceases.

Determinants of circulatory failure. The problem of standardization. Our experience emphatically suggests that the development of irreversible circulatory failure after hemorrhage is not determined by the percentage volume of blood lost. Some animals develop shock after withdrawal of a blood volume equal to 3 per cent body weight or much less if extensive operative procedures are carried out at the same time; whereas, others can be restored to normal after a volume equal to 5.0 to 5.6 per cent body weight has been withdrawn. It is true that animals may die as a result of such large hemorrhages but since they can be revived by prompt substantial infusions of their own blood, such death cannot be attributed to shock.

All our evidence suggests that the degree and duration of post-hemorrhagic hypotension are of dominant importance. Our initial experience coincided with that of others (for review *cf.* 1), *viz.*, that many dogs can be kept at pressures between 50–60 mm. Hg for 3 or 4 hours and will recover through subsequent infusion. In order to produce a state which cannot be improved more than temporarily by substantial infusions, arterial pressures must be really low for an effective interval; indeed, generally it must be so low that the circulation is barely maintained and failure of the heart and respiration are threatened.

In order to gain some idea as to the minimal effective intensity and duration of hypotension which causes shock we submitted different dogs to an initial period of moderate hypertension followed by a second period of extreme hypertension. The following procedure was developed. A dog was bled rapidly (ca, 50 cc./min.) up to 2 or 3 per cent of his body weight, but in no event was mean arterial pressure allowed to fall below 60 mm. Hg. After a recovery period of at least 15 minutes, the animal was bled slowly (6–20 cc. per min.) until arterial pressure had been reduced to about 40 mm. If the pressures rose above 60 mm. the dog was cautiously bled a little more. In this way an *initial period* of moderate hypotension (averaging 50 mm.) was maintained for an hour or more. Then the animal was again bled slowly (ca, 6–10 cc./min.) until mean pressures were reduced to about 30 mm., cautiously bleeding a little more whenever pressures approached a 40 mm. level, or infusing a little blood if the heart began to slow or respiration threatened to fail. In this way an *extreme hypotension* at a level of approximately 30 mm. mean pressure was produced.

Our results which are analyzed in detail elsewhere (2) indicate that an initial period of *moderate* hypotension, lasting 90 minutes, followed by a period of *extreme* hypotension for 45 minutes always resulted in irreversible failure. These durations exceed the minimal requirement. Similar periods of moderate and extreme hypotension of 60 and 30 minutes, respectively, were adequate in

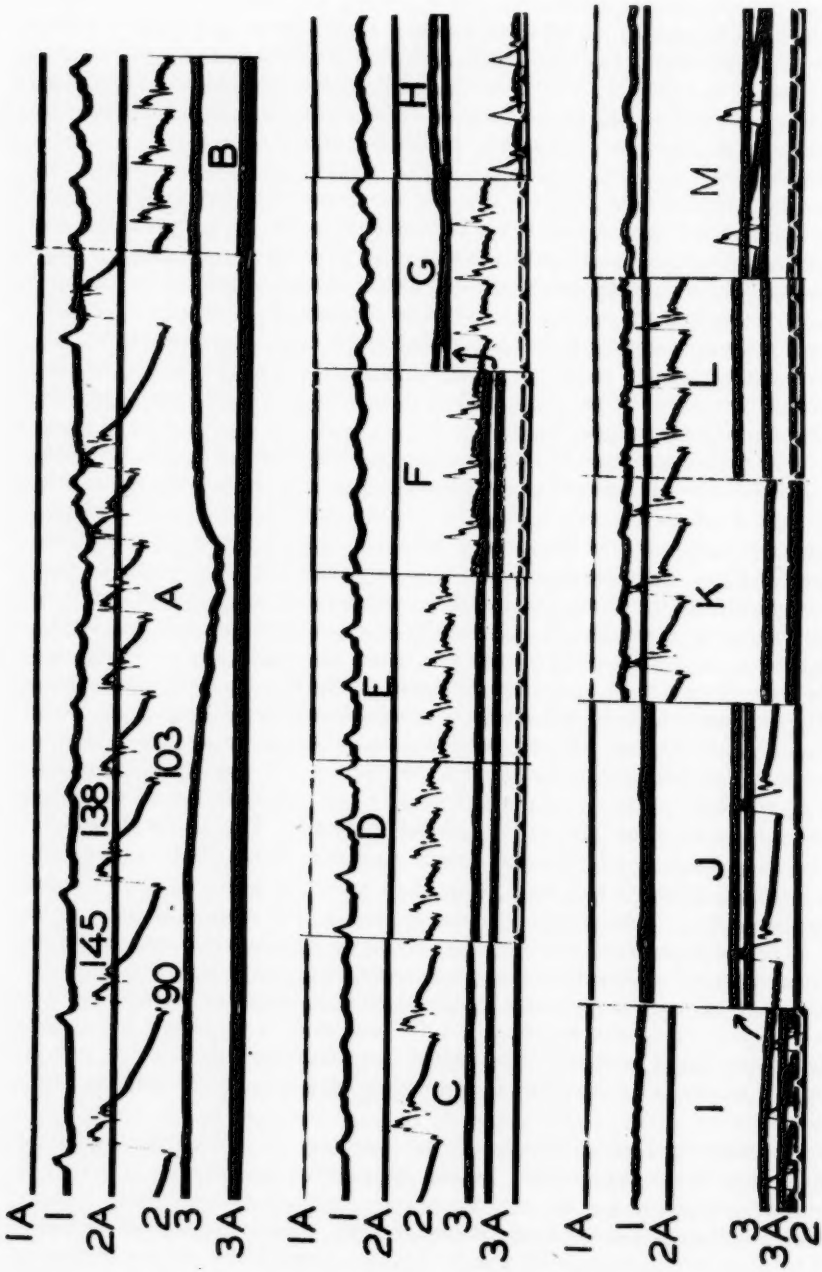

FIG. 4

some animals, but not in the majority. The minimal affective intervals that may prove useful in developing a standardized technique for producing hemorrhagic shock lie somewhere between these ranges.

Correlations between dynamic criteria of shock and pathological changes in the viscera. Autopsies were performed in over 90 per cent of our animals. In control animals that died quickly as a direct result of large rapid hemorrhages, pallor of the stomach, gut, mesentery and omentum was a conspicuous feature. The mucosal surfaces were pale and either dry or slightly moist. The spleen was small, hard, firm and rough. Other organs showed no conspicuous changes.

Animals that survived a period of low pressure and were restored by blood infusion for several hours were killed by asphyxia. As a rule, they showed no differences from normal dogs; a few showed scattered petechial hemorrhages in heart and intestinal mucosa, but on the whole the mucosa was normal or very slightly swollen.

Animals in which blood infusion after prolonged hemorrhagic hypotension was only of temporary benefit and which died from progressive circulatory failure usually displayed quite a different pathological picture in the upper intestines. Upon opening the abdomen the mesenteric vessels were not particularly engorged, the liver appeared normal and the spleen was variable, sometimes large and congested, occasionally normal, but generally small and shrunken. The peritoneal cavity contained small amounts of serous fluid but ascites could not be said to exist. On opening the jejunum and ileum, striking pathological changes were generally revealed. In the milder cases, the lumen always contained abundant fluid, heavily tinged with dark bile. The mucosa was swollen and congested and sometimes petechial hemorrhages were noted. In severe cases, the lumen contained considerable quantities of bloody fluid which was signaled in several experiments by passage of bloody stools or staining of a rectal thermometer before death. The mucosa was thick, edematous and congested and contained many bleeding areas. In a few cases, longitudinal striae of congested vessels were noted. With a single exception, in which the gastric mucosa was chiefly involved, the congestive and hemorrhagic condition stopped abruptly in the region of the duodenal bulb; the gastric mucosa appeared pale by contrast. Nor was the mucosa of the ileum or large intestine involved.

Fig. 4. Central venous pressure (1), central arterial pressure (2), intrathoracic pressure (3). Base lines for same, (1A), (2A), (3A).

	Time	H.R.	B.P.		Time	H.R.	B.P.
A	10:43—see record			H	12:05	190	48/25
B	10:55	194	112/89	I	1:35	150	45/25
C	11:10	112	130/89	J	2:14	75	52/20
					(arterial curve and		
D	11:21	214	106/90		base line raised		
E	11:31	198	95/80	K	2:46	144	135/95
F	11:43	182	73/55	L	2:56	182	118/98
G	12:00	185	68/57	M	3:13	91	60/30

Examination of other organs occasionally revealed scattered petechial hemorrhages on the cardiac and serous surfaces; but these were inconstant. Gross evidence of pulmonary edema never existed. With two exceptions, pericardial fluid was not increased. In short, the pathological changes which correlated with the dynamic criteria were limited fairly strictly to the duodenum and jejunum. They were practically the same as those described by Erlanger and Gasser (3) in shock produced by various procedures.

The question naturally arose whether the mucosal changes preceded or followed reinfusion of blood. To study this problem, a loop of upper intestine was exteriorized in three animals, opened and observed during the whole experiment. The mucosa became more pale during the course of hemorrhages, but while pressures were low cyanosis, congestion and edema developed in the exposed mucosa surface. However, since newly opened sections of gut appeared normal, these effects were apparently caused by exposure rather than hypotension. In three other animals, a duodenal loop was exteriorized *at the end* of a low pressure period regarded as sufficient to cause irreversible circulatory failure, but before reinfusion of blood. The lumen contained fair amounts of secretions and in two experiments a liberal secretion from the pancreatic papillae was observed. The mucosa did not appear congested or swollen in two of these animals, but slight congestion and thickening of the mucosa was present in the other. With injection of blood the mucosa rapidly became darker and more swollen and began to bleed. As circulatory failure developed the luminal contents became bloody. In one animal bloody fluid from other regions was periodically expelled from the opened gut by peristaltic contractions.

The correlation of these pathologic and circulatory changes is interesting, but their meaning is not entirely clear. For example, it remains an enigma why following generalized reduction in blood flow these striking changes are localized so strictly in the upper gut. Apparently, the vessels of this region are particularly vulnerable so that a period of effective hypotension causes some change in the minute vessels of the mucosa and they become intensely congested or even rupture with reinfusion of blood. That such changes are the result rather than the cause of the initial hypotension cannot be argued in this case. That they account wholly for the subsequent circulatory failure after restoration of blood to the circulation must not be inferred too hastily.

Admittedly, the congested bleeding mucosa demobilizes large quantities of blood and additional volumes of fluid are lost into the lumen, but such gross observations do not prove that the effective circulating volume is diminished sufficiently to explain the progressive failure which supervenes.

Venous pressures during post-hemorrhagic hypotension and hemorrhagic shock. The conception that decreased venous return causes a reduction of effective venous pressure and that the latter is responsible for decreased systolic discharge is the keystone of all modern conceptions of shock. This postulate, originated by Y. Henderson (4), has been supported by investigations of one of us (5). However, it could be established in a more crucial manner and it is not a necessary corollary that irreversible circulatory failure only results when

venous pressures have fallen so far that ventricular filling is below a critical level. (For recent review, see Wiggers (1).)

Accurate measurement of venous pressure during the course of a long experiment is not the simple procedure that it is generally thought to be. The following facts need to be kept in mind:

1. We must record central venous pressures within the thorax, not peripheral venous pressures which are subject to local changes in flow.

2. When central venous pressures are recorded by sensitive optical manometers from a sound introduced into the right auricle or superior vena cava, it is found that such pressures are far from constant. A calibrated record is shown in figure 5–III. In such curves the cyclic variations during the short expiratory apnea can vary as much as 135 mm. H_2O, and during inspiration these values are 60 to 180 mm. H_2O lower.

3. The mean of the cardiac fluctuations shown in figure 5–III would probably be an adequate index of average filling pressures under normal conditions. Unfortunately, a low-frequency water manometer (damped or undamped) only approximates such readings, owing to the complicated interferences of cardiac and respiratory variations.

4. Since it is time-consuming to enlarge planimeter and integrate optical records such as are shown in figure 5–III, it is more practical to measure these curves at some constant point at all times. The dilemma arises as to which point to choose. Previously, one of us (6) had suggested the pressure value at point X because it represents the pressure under which ventricular filling begins. However, comparison of mean integrated values and pressures at other moments of the cycle, such as X–Y, Z in figure 5–III in several of our experiments, indicated that the pressure at the extreme end of diastole (Z) showed the best correlation. Measurement at this telediastolic point (Z) can also be defended on the grounds that it takes into account the varying vigor of atrial contraction during the course of hemorrhage and shock (*cf.* upper curves of figs. 1, 2, 3) and represents the stretching pressures in the common atrial and ventricular cavities at the very end of filling. We therefore chose this point of measurement (Z) in all of our curves in comparison of recorded venous pressures.

5. Such central venous pressures are recorded by manometers which are balanced against a constant atmospheric pressure; but within the atrium and large veins they are balanced against varying sub-atmospheric intrathoracic pressures. Records of right ventricular pressure show that these variations in intrathoracic pressure are communicated through the right ventricular as well as the atrial walls (7, 8). Therefore, in order to obtain an idea of pressures that are effective in filling the ventricles within the closed chest, it is important, as Y. Henderson and Barringer (9) first emphasized, to measure the algebraic difference between intrathoracic and actual intravenous pressures, *i.e., the effective venous pressure.*

6. Since the tonus of respiratory muscles or mechanical factors may conceivably change intrathoracic pressure during prolonged experiments, it is necessary to measure each pressure separately—not differentially, as was done

by one of us (5)—in order to determine whether changes in effective venous pressure are due to alterations in intrathoracic or actual venous pressures. In twelve experiments such measurements were made, as far as possible, during the period of brief expiratory apnea and, of course, always at synchronous times.

RESULTS. Changes in recorded venous pressures are shown in the reduced segments of original optical records in figures 1, 2 and 3. The measured telediastolic or Z-pressures, together with intrathoracic pressures and calculated effective venous pressures, are plotted in curves of figure 5. In these, the data indicated by Roman numerals (I, II, IV) correspond to data obtained from records of figures 1, 2 and 4 respectively, and the letters to the segments of records in these figures.

The plots of figure 5, I show *1*, a distinct but temporary fall in recorded and effective venous pressures (lower curve) during a rapid loss of blood (A–B); *2*, a prompt recovery (C); *3*, a progressive decline during the second lower hemorrhage (E–I), and *4*, a fair correspondence between changes in recorded and effective pressures, because intrathoracic pressure (IP) changed gradually and not extensively. The latter was an uncommon, not the usual result, as subsequent curves will show.

The plot of figure 5, II shows that the intrathoracic pressures (IP), measured during expiratory apnea, changed considerably during the course of the experiment, and that these changes materially altered the calculated effective venous pressures (EVP); *2*, that as a result of a slow hemorrhage (A–E), effective venous pressure declined from 78 to 32 mm. H_2O; *3*, that the magnitude of the decrease was greater than that of the recorded venous pressure, because intrathoracic pressure became less negative; *4*, that, consonant with current opinion, the decline of blood pressure and deterioration of pressure pulse contours shown in segments B–E of figure 2 are related to the fall in effective venous pressures; *5*, that toward the end of a slow hemorrhage and for approximately an hour after its cessation (E–F), recorded venous pressure (VP) and calculated effective venous pressures (IVP) were approximately restored to normal values, while arterial pressures continued to fall and the form of the pressure pulses deteriorated; *6*, that reinfusion of all the blood withdrawn by previous hemorrhage (J–K), elevated the recorded but not the effective venous pressure; *7*, that the arterial pressure again fell and the pressure curves began to deteriorate (cf. fig. 2, K–L) despite the fact that recorded and effective venous pressure remained at normal levels for nearly an hour; *8*, that the final collapse of arterial pressure pulses (L–P) was associated with slight decline of recorded and effective venous pressures, but *9*, that when the circulation was very low (*Q*), as well as immediately after death, recorded and effective venous pressures equaled levels found at the start of the experiment. The trend of venous pressures shown in this plot was typical of those in eight other experiments.

The curves of figure 5, IV illustrate venous pressure changes characteristic of four other experiments. This plot shows *1*, that, as anticipated, actual and effective venous pressures fell markedly during the initial large hemorrhage (A, B), and partially recovered therefrom shortly after hemorrhage had ceased

(C); *2*, that a second but slower hemorrhage (C, G) reduced recorded and effective venous pressures fairly permanently,—while arterial pressures remained

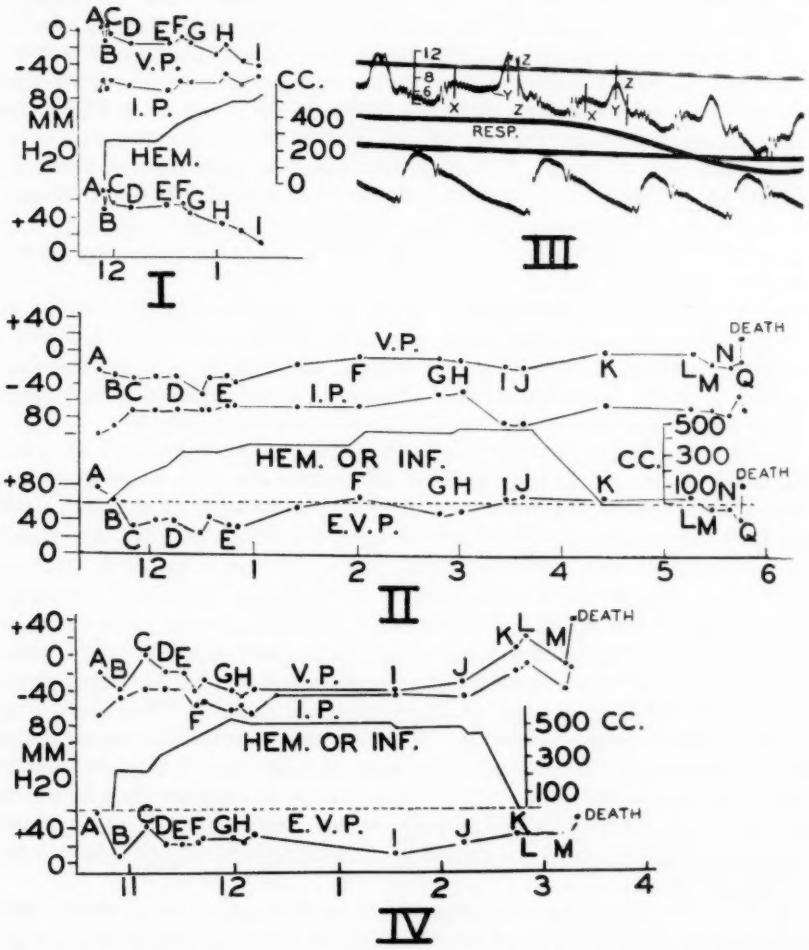

Fig. 5. I, II, IV, plots of actual venous pressure (V.P.), intrathoracic pressure (I.P.), and effective venous pressures (E.V.P.) plotted in relation to hemorrhages and infusion. From curves represented by segments of records in figures 1, 2 and 4. III, original tracing of venous, intrathoracic and subclavian pressures showing variations of venous pressures. Description in text.

low for the next two hours, and the pressure pulses stayed abnormal (H, I); *3*, that the serious circulatory failure shown in segment J of figure 3 was attended by a slight increase in effective venous pressures between I and J, due to a rise of recorded venous pressure; *4*, that reinfusion of the lost blood raised recorded

pressure greatly and effective pressure but slightly (K, L); *5*, that the subsequent sudden decline of arterial pressure and slowing of the heart (*M*) were attended by a reduction in recorded, but no change in effective venous pressures.

Deductions. In the first place, withdrawal or reinfusion of blood—particularly if fairly rapid—causes significant changes in intrathoracic pressure, which often make determinations of actual venous pressure deceptive as regards quantitative and even directional trends. As illustrated in figures 5, II, IV, reinfusion of blood—as may be expected—raises intrathoracic pressure, i.e., renders it less negative. However, as these graphs also show, withdrawal of blood often has the same effect. It is tempting to assign this to a reduction in tonus of respiratory muscles during expiration, particularly as Gesell and Moyle (10) recorded a slight lengthening of skeletal muscles during hemorrhage; but we have no direct proof for this.

In the second place, no question exists that recorded and effective central venous pressures drop significantly soon after the start of a large hemorrhage equal to 2 per cent of the body weight or more, and to judge from pressure pulses systolic discharge decreases at once. However, after cessation of such hemorrhages venous pressures rise promptly, often returning to normal.

In the third place, during the period when arterial pressures have been stabilized at low levels by one large and successive slow hemorrhages, the recorded and effective pressures remain low in the minority of animals. In the greater number, illustrated by graphs of figure 5, II, both recorded and effective venous pressures rise, often to normal levels. It seems significant that this recovery is not associated with a similar improvement in arterial pressures or in the form of the arterial pulse curves. Similarly, after all the withdrawn blood has been reinfused following an effective period of low pressure, venous pressures rise and in the majority of animals remain at normal or supernormal levels. Nevertheless, arterial pressures decrease again and the form of the arterial pressure pulses deteriorate. While in some animals ultimate circulatory failure parallels decline in venous pressures, the fact that in a greater number similar failure occurs without such decline makes it apparent that failure can be due to other causes in hemorrhagic shock. Henderson's dictum, "venous pressure is the fulcrum of the circulation," certainly does not hold in this type of hemorrhagic shock.

In the fourth place, regardless of previous trends, recorded and effective venous pressures always increase when the heart begins to slow during terminal stages, apparently because of stasis. This emphasizes again that venous pressures are determined by at least two factors: *1*, the volume of venous return, and *2*, the volume pumped away by the heart. It is therefore conceivable that the return of venous pressures to or toward normal while arterial pressure declines may be due to the fact that a reduced venous return is nicely counterbalanced by the decreased cardiac output. However, if this be true, primary myocardial depression must be assumed.

Hematocrit determinations. These were originally instituted because, in our experiments, it was necessary to flush various cannulae of optical manometers periodically in order to assure ourselves of their complete patency. During the

course of long experiments considerable quantities of heparinized Ringer's solution were thus introduced which might be expected to increase the natural hemodilution following hemorrhage. Consequently, in nine experiments we attempted to gauge the extent of such dilution and its effect on dynamic changes. To our surprise we found no unusual decrease in hematocrit readings during the period of post-hemorrhagic hypotension, and a slight increase in cell/plasma ratios after reinfusion. Typical results are given in abridged table 1.

TABLE 1

SAMPLE NO.	TIME	HEMATOCRIT	B.P.	REMARKS
Dog K-17, 10 kilos, morphine, sodium barbital				
1	9:55	43.5	120	Control
2	11:50	30.8	60	13 min. after total 475 cc. hemorrhage
3	1:20	48.2	80	After 90 minutes' hypotension and reinfusion
4	2:00	47	58	
5	3:25	44	45	Natural circulatory failure
6	4:12	44.4	20	Death followed
Dog K-30, 10 kilos, morphine, sodium barbital				
1	11:14	45.5	135	Control
2	11:45	39.6	50	After 90 minutes of moderate hypotension
3	12:37	41.0	35	
4	1:28	42.3	40	
5	2:22	45.9	35	
6	3:27	48.4	60	After reinfusion
7	4:25	46.2	35	Decline and death
Dog K-20, 11 kilos, morphine, sodium barbital				
1	10:31	47.3	155	Control
2	10:58	39.4	60	26 min. after 550 cc. hem.
3	11:27	36.9	40	24 min. after 40 cc. hem.
4	12:05	39.9	30	33 min. after reinfusion 500 cc.
5	12:55	41.5	30	43 min. after reinfusion 550 cc.
6	1:56	52.0	118	28 min. after reinfusion all blood
7	2:47	51.9	120	
8	3:21	51.9	120	2 hrs. after infusion Apparent recovery, no gut changes, etc.

Since the processes leading to shock were apparently operating during the post-hemorrhagic hypotensive period while hemodilution existed, our experiments lend little support to the idea that development of shock is necessarily related to hemoconcentration.

Plasmapheresis experiments. In order to test further whether shock developed more quickly and certainly when loss of blood volume was accompanied by hemoconcentration, six experiments were performed in which the blood withdrawn

was centrifuged and the red corpuscles returned according to standard procedures. Our results showed that after each reinjection of a substantial volume or corpuscles, the cell/plasma ratios and presumably the blood viscosity and peripheral resistance increased. Such injections made it more difficult to reduce the arterial pressures and hence to induce a state of shock by withdrawal of equivalent circulating volumes. It was our impression that hypotension rather than hypovolemia *per se* is the dominant factor in shock following hemorrhage. While we hesitate to draw general conclusions with regard to the contributing rôle that spontaneously developing hemoconcentration may play in development of other types of shock, it was our impression that artificial hemoconcentration retards rather than hastens the development of hemorrhagic shock.

Discussion. *The experimental criteria of shock.* While the syndrome now called "shock" probably involves disorganization of systems other than the circulation, a progressive circulatory failure which becomes irreversible without treatment is unquestionably a dominant feature.

Our experiments indicate that while the state of hypotension which follows severe hemorrhage is not necessarily equivalent to shock, it can, if sufficient in degree and duration, lead to such a state, and apparently as a result of prolonged hypotension rather than hypovolemia *per se*. Our experimental and pathologic evidence suggests that both the peripheral circulation and heart contribute, in different proportion in different animals, to the phenomenon of irreversibility.

Unfortunately, no criterion has yet been discovered by which the existence of such an irreversible state can be determined. Neither changes in arterial or venous pressures nor characteristics of arterial pressure pulses offer any criterion which distinguishes between hemorrhagic hypotension or shock. Irreversible shock develops under states of hemodilution or hemoconcentration. The only reliable criterion seems to be whether or not an animal recovers after substantial infusions of whole blood, and possibly of plasma or serum.

While we recognize the clinical disadvantage of restricting the term to hopeless terminal conditions which cannot be improved more than temporarily by infusions, we feel that such limitation would correlate experimental and pathological criteria, and it would focus greater attention on the nature of irreversible mechanisms and efforts to counteract them. Above all, it would tend to establish among laboratory investigators more rigid criteria for testing agents and measures reputed to have therapeutic value. Maintenance of arterial pressure at low levels for several hours as a result of any procedure is not a sufficient index that irreversible circulatory failure exists. The use of such animals for the assay of various therapeutic agents does not constitute a crucial test. If, on the contrary, such agents prove effective after an animal has failed to respond to substantial infusions, some claim of efficacy is allowable.

SUMMARY

By the expedient of regulated hemorrhages, dogs anesthetized with sodium barbital, amytal or chloralosane were kept in a state of severe hypotension for

varying intervals, after which all the withdrawn blood (heparinized) was reinjected. Central arterial, central venous and intrathoracic pressure changes were recorded throughout the experiments.

A continuing state of post-hemorrhagic hypotension is not necessarily equivalent to shock, for in many animals *a*, arterial pressures and pulses were restored to normal for many hours by reinfusion of the withdrawn blood even when such animals were on the verge of cardiac or respiratory failure, and *b*, the viscera showed no pathological changes at autopsy.

If, however, both the intensity and duration of the post-hemorrhagic hypotension were great enough, *hemorrhagic shock* developed for *a*, the condition was only temporarily benefited by generous infusion of blood, and *b*, the duodenal and jejunal mucosa was generally edematous, congested and bleeding with presence of excessive fluid and blood in the lumen. Other organs showed no consistent pathological changes.

While an elusive "resistance factor" interferes with attempts to standardize the procedure for experimental purposes, our results suggest that the greatest hope lies in creation of a preliminary period of moderate hypotension (*ca.* 50 mm.) followed by a shorter period of extreme hypotension (*ca.* 30 mm.). Using such a scheme, the minimum effective durations for these respective stages were found to be less than 90 and 45 minutes and more than 60 and 30 minutes in our trials.

We have discovered no new dynamic criteria which enables us to determine whether a period of post-hemorrhagic hypotension will be followed by failure or recovery on reinfusion. Indeed, hemorrhage equal to 3 to 4 per cent body weight can produce all the changes in arterial pressure pulses seen in most severe shock due to other causes (5). Irreversibility after substantial infusion, admittedly unsatisfactory as a practical guide, is unfortunately the only reliable one at present.

Circulatory failure following reinfusion of all withdrawn blood developed despite an adequate blood volume and regardless of hemodilution or hemoconcentration. Augmented hemoconcentration induced by plasmapheresis had no discoverable accelerating action. The factor which precipitates the irreversible state of hemorrhagic shock resides in the cardiovascular system. Reduction of effective venous pressure did not account for the failure of arterial pressures in the majority of our animals. Since, in the majority, effective venous pressures were at or above normal levels during the post-infusion decline of arterial pressures, the deterioration of arterial pressure pulses must be due to impairment of the heart, changes in the aorta and its elastic branches and/or in the resistance at the periphery of the arterial tree.

The supervention of cardiac alternans during early periods of post-hemorrhagic hypotension, the later tendency toward progressive slowing, the poor response to rapid infusions and the terminal rise of venous pressures suggest operation of a cardiac precipitating factor in some of our animals.

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CARDIAC AND PERIPHERAL RESISTANCE FACTORS AS DETERMINANTS OF CIRCULATORY FAILURE IN HEMORRHAGIC SHOCK[1]

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In a previous communication (1) we reported that the state of hypotension which follows severe hemorrhage is not necessarily equivalent to that of shock but that the progressive circulatory failure which supervenes after an effective period of such hypotension and the reinfusion of all the withdrawn blood warrants the designation, *hemorrhagic shock*. In some animals such a state developed without a reduction or even with an increase in central venous pressures, which suggested that failure of the myocardium might be a factor.

In a recent review (2) one of us pointed out that a number of investigations strongly indicate that arterioles of certain regions, particularly those of the limbs, salivary glands and spleen, constrict but that this conclusion is by no means unanimous. Since total peripheral resistance (TPR) is determined by numerous regional resistances arranged in parallel ($1/TPR = 1/R_1 + 1/R_2 - - - 1/R_n$), changes of resistance in one territory may not give a correct index as to the extent that changes in TPR favor or antagonize the progressive downward course of arterial pressure in shock.

This report attempts *1*, to evaluate the state of myocardium and TPR during prolonged post-hemorrhagic hypotension and following reinfusion of all withdrawn blood, and *2*, to assess the relative importance of changes in venous pressure, myocardial responses and TPR in the promotion or prevention of irreversible circulatory failure.

METHODS. Optical records were made periodically of aortic and right atrial pressures together with volume changes of the ventricles. The methods employed for the latter are described elsewhere (3). In addition, records of mean femoral pressure and changes in ventricular volume were recorded continuously on a slowly moving smoked drum. The optical volume curves were used for evaluating the mechanisms of ejection and filling; the drum records, to estimate changes in systolic discharge and minute output quantitatively. For this purpose, the records were calibrated frequently, while the heart was beating in the cardiometer, by withdrawing or introducing known volumes of air (fig. 1, D). This also established absence of leaks.

Total peripheral resistance was calculated by the formula,

$$TPR = \frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec.}} = \frac{\text{dynes} \cdot \text{sec.}}{\text{cm.}^5}$$

We shall refer to these as absolute units (A.U.).

[1] Supported by a grant from the Commonwealth Fund.

Critique of methods. The value of our conclusions depends largely on the accuracy with which qualitative and quantitative changes in ventricular filling and output are determined by cardiometric methods. A brief evaluation of available methods and the precautions that must be exercised is therefore indispensable. Cardiometric registrations introduced by Y. Henderson have proved particularly valuable in analyzing the mechanisms of cardiac filling and emptying when directional changes occur within short periods. Even in such studies, technical errors have occurred and misinterpretations have arisen as Y. Henderson (4) and later Wiggers and Katz (5) have pointed out. For example, the end of ejection and the magnitude of the stroke cannot always be determined precisely without simultaneous records of aortic pressures.

When the ventricular volume curves are used quantitatively in experiments of long duration, errors easily creep in despite meticulous efforts to fix the cardiometer diaphragm with respect to the A-V junction. Whenever the capacity of the ventricles and the circumference of their base alter considerably, as in our experiments, it requires a good deal of judgment and practice to maintain an ideal placement of the heart and a perfect fit of the rubber diaphragm throughout the experiment. When the circumference of the ventricular base decreases after severe hemorrhage, leaks tend to develop; when it gets larger after infusion a mechanical A-V stenosis may occur.

We were able to minimize these difficulties by allowing the open margin of the rubber diaphragm to form a rolling cuff about the ventricular base. This cuff tended to be taken up as the circumference decreased during hemorrhage and to roll up as the heart dilated during infusion. As a rule, the decrease in diameter during bleeding did not cause any leak, as verified by calibration tests. However, when the heart dilated as a result of a rapid reinfusion, a slight stenosis occasionally developed. Fortunately, this was evidenced by an inordinately large atrial wave in the right atrial pressure curve and an altered mode of ventricular filling. Such an extreme effect is illustrated by comparison of curves A and B of figure 1. In curve A, obtained after hemorrhage, atrial pressure decreases with the onset of ventricular filling at Z and the subsequent atrial contraction causes a moderate elevation of atrial pressure (A). In curve B, obtained shortly thereafter, subsequent to a large infusion of blood, the pronounced rise of pressure during atrial contraction is chiefly responsible for ventricular filling; indeed vibrations presumably due to an associated murmur are superimposed on the volume curve. Such curves must obviously be discarded. Fortunately, such tendency to mechanical stenosis disappears quickly. Furthermore, after we recognized the possibility of this artifact, it could generally be avoided, as shown in curve C, by grading the infusion rate to the capacity of the ventricle to move blood forward. However, if volume curves show a more gradual filling rate toward the end of an experiment, the question often needs to be settled whether this was a natural phenomenon or was due to development of A-V constriction as the ventricles progressively dilated. The amplitude of the optical atrial pressure pulse is always a useful guide in such cases. If the pressure wave due to atrial contraction is extremely large, a stenosis effect must

be suspected; if it is normal, stenosis is not the factor. Further, if an even greater ventricular dilatation by a preceding infusion such as occurred in figure 4, curve 23A, results in a volume curve of normal design, the reduced filling rates of succeeding curves 25 and 26 are probably not occasioned by an artificial A-V stenosis.

Another artifact is introduced when the ventricular basal movement alters under different states of filling. It frequently happens that these effects are of

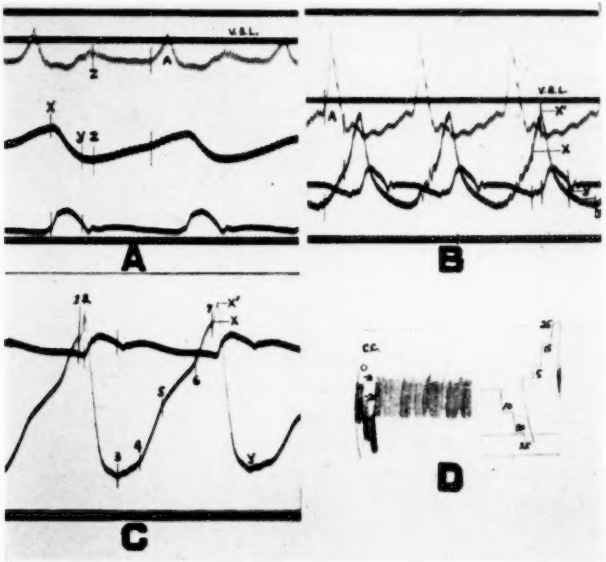


Fig. 1. *A*. Atrial pressure (upper) volume curve (middle) and aortic pressure pulse (lower) after hemorrhage. V.B.L., base line for volume curve. Description in text.

B. Same after infusion of blood causing distortions of volume curve due to A-V stenosis and movement of cardiac base, rendering curves worthless. Description in text. Note that ventricular volume curve moved down to correspond with shift in base line, V.B.L.

C. Accurate volume curves after infusion with but slight X-X^1 artifact; 1–2 isometric contraction; 2–3 ejection; 3–4 protodiastole and isometric relaxation; 4–5 rapid filling; 5–6 diastasis; 6–7 atrial contraction.

D. Drum record corresponding to curves of *B*, showing no signs of imperfect recording revealed by optical curve. Also illustrates method of calibration, with and without beating heart in cardiometer.

such a nature that the ventricles are pushed into the cardiometer during isometric contraction and pulled out during isometric relaxation with the result that the tops and bottoms of cardiometer records do not represent the size of the ventricles at the beginning and end of ejection. This is illustrated in a greatly exaggerated way in figure 1*B*. Homonymous points of the arterial pressure pulse and volume curves show that the volume curve increases tremendously during isometric contraction (X-X^1) and continues to decrease during

isometric relaxation (Y-Y^1). In such records X-X^1 and Y-Y^1 are artifacts which result in wrong values if the whole stroke is used to measure systolic discharge.

Such artifacts can of course always be recognized in optical tracings taken simultaneously with aortic pressure curves; but in slow kymograph tracings, such as are shown in figure 1*B*, they are concealed. Unless recognized, this artifact obviously introduces errors in quantitative estimations of cardiac output, which are preferably made from slow kymographic tracings because these can easily be calibrated while the heart is beating in the cardiometer (fig. 1*D*). However, owing to the size of optical curves desirable, satisfactory calibration cannot be made under similar circumstances within the limits of a 12 cm. lens of the photokymograph.

We, therefore, used optical records not only to study qualitative changes in filling and ejection, but also to check the reliability of kymograph tracings used for quantitative measurements of cardiac output. We now realize that failure to make such checks was perhaps responsible for discrepancies encountered by Dingle, Kent et al. (6) in calculating TPR.

This method for calculating stroke and minute volumes is laborious and not as precise as we should like; but data so derived probably compare favorably with those obtained by gasometric and various physical methods which are equally unsatisfactory. Like these, our calculations are sufficiently accurate to show directional trends of quantitative changes.

Finally, in using cardiometric methods for studying cardiac output, the chest must be opened and artificial respiration instituted. Such ventilation must be deep enough to avoid anoxia and yet not so intense that it induces acapnia or impedes pulmonary blood flow. However, even with attention to this detail (cf. 2), also avoidance of significant hemorrhage and severance of intercostal nerves, opening of the chest *per se* creates a condition that has many resemblances to effects of mild hemorrhage. It is our experience also that many animals thus operated tolerate much less loss of blood before pressures are stabilized at very low levels; but exceptions occur. However, it is also true that unless additional insults occur (e.g., hemorrhage, trauma, etc.), the circulatory state tends to improve rather than deteriorate. Consequently, if the interpretation of results is properly tempered, considerable information may still be obtained regarding superimposed shock-producing agents.

Procedures. As in previous experiments (1), dogs under morphine-sodium barbital anesthesia were bled until a period of marked hypotension was maintained for 30 minutes to 2 hours. At the end of such a period, the withdrawn blood was reinfused and the course of dynamic events followed for 4 to 5 hours, unless death occurred earlier.

Results. In such experiments there are two physiologically important periods: *1*, the period of post-hemorrhagic hypotension during which functional damage is initiated, and *2*, the post-infusion period during which the effects of any damage become manifest. Experiments on 11 dogs consistently showed a decrease in stroke and minute volumes of the ventricles during each of these

periods. But the manner in which this reduction was accomplished, the changes in TPR which occurred and the effects on arterial pressures and pressure pulses were strikingly different in different experiments.

An analysis of these variations requires a fairly detailed consideration of at least three of our experiments. In order to conserve space, the data have been reduced to three charts (figs. 2, 3, 4), each of which shows plots of the time and extent of bleeding and reinfusion, changes in heart rate, mean arterial pressure, total peripheral resistance in absolute units, cardiac output per minute, together with transcribed tracings of selected ventricular volume curves and

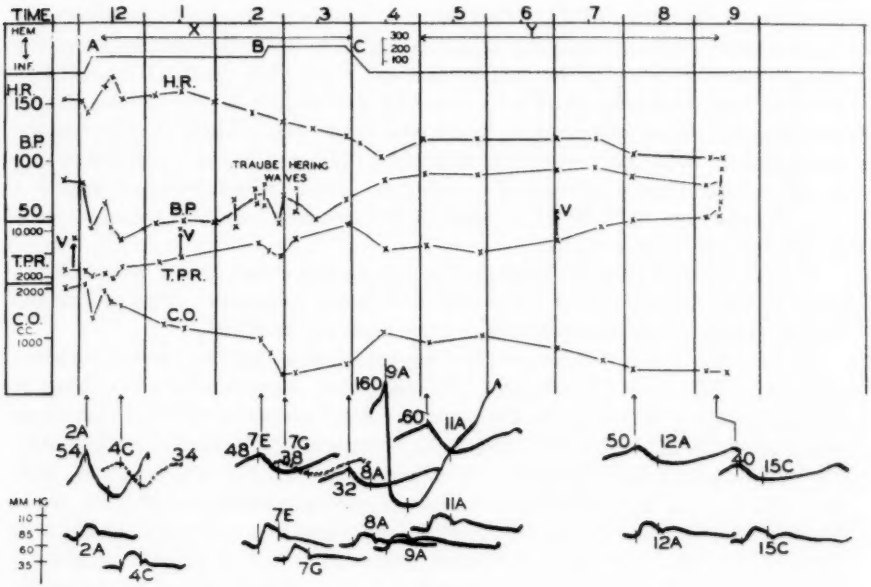


Fig. 2

aortic pressure pulses. Venous pressures are directly inscribed with each volume curve.

Figure 2 illustrates results from a dog in which arterial pressure was kept below 50 mm. Hg for 1½ hours by a first hemorrhage of 170 cc. (A), and when arterial pressures increased thereafter the animal was bled another 90 cc. (B), but this only reduced pressures to 50–70 mm. Hg for another half hour. The span of the post-hemorrhagic period is indicated by the line, X. While mean pressure was gradually rising, all the withdrawn blood was reinfused at C. Five and a half hours later (ca, 9 p.m.), mean arterial pressure was as high as at 11 a.m., and the heart rate had decreased from 150 to 100 per minute. Venous pressure had not changed significantly at the end. By no easily determinable criterion could shock be said to be impending and we labeled this a

recovery animal. Nevertheless, an analysis of changes in stroke volume, minute output and total peripheral resistance suggests that such a process may have been in progress. It stresses the need for care in making a prognosis on the basis of sustained arterial pressures alone.

During the hypotensive period following the first hemorrhage, we note that cardiac minute output decreased progressively and that this was followed by a further decrease after the second hemorrhage. The volume curves below show a typical decrease in diastolic filling and stroke, the trend corresponding to reduction in venous pressures. Nevertheless, mean arterial pressure and pressure pulses showed considerable recovery due to the progressive increase in total peripheral resistance (TPR). This increase was never maximal, as shown by an additional increase in mean pressure and total peripheral resistance following stimulation of the central left vagus (marked V on TPR curve). In addition, marked Traube-Hering variations occurred just before and after the second hemorrhage. With reinfusion of blood, the heart rate slowed—the customary finding in our laboratory—but the tremendous increase in venous pressure and stroke volume shown in curve 9A served to elevate mean pressure despite a prompt reduction in TPR. At autopsy, the duodenal mucosa appeared congested but not hemorrhagic.

During the post-infusion period (Y), the curves clearly show that arterial pressure was sustained by an increasing TPR, while ventricular filling and stroke volume progressively decreased and venous pressures declined.

Comparison of the volume curves recorded at essentially equal venous pressures, e.g., 2-A, 11-A, 12-A and 4-C, 7-G and 15-C suggest progressive diminution in stroke with respect to comparable venous pressures, but the variations might be within the limits of experimental registration. Certainly, in comparing the reactions of more proximate recordings, e.g., 7E, 7G, 8A, 9A, 11A, 12A and 15C, it could be said that the changes compare qualitatively with those described by Starling (7) in the heart-lung preparation and by Wiggers and Katz (5) for the controlled circulation preparation. In a general way also, we may say that cardiac output during the post-infusion period decreased in part through slowing of the heart and in part through reduced stroke volume consequent to diminishing venous pressures, and that arterial pressure was maintained by compensatory increase in TPR. In other words, an incipient state of circulatory failure was developing after the manner first described by Y. Henderson (8).

A similar course was followed in five other experiments. In two of these, TPR remained increased during the post-infusion period, nevertheless, they terminated fatally. In two other fatal experiments, no significant changes in TPR occurred throughout the experiment, and in one it alternately increased and decreased, leveling a little above normal toward the end. Such results indicate that hemorrhagic shock can occur regardless of whether TPR is high, normal or low.

The experiment charted in figure 3 illustrates a number of different features. To judge from cardiac output data and the good arterial pressure and pulse at

the start, this animal appeared in much better condition initially. However, withdrawal at A of only 180 cc. (1.8 per cent body weight) caused so precarious a fall of blood pressure that 50 cc. was promptly reinfused at B. Following this, arterial pressure was maintained above 50 mm. for about 30 min., and thereafter progressively declined to about 35 mm. Hg, at which level it remained for about

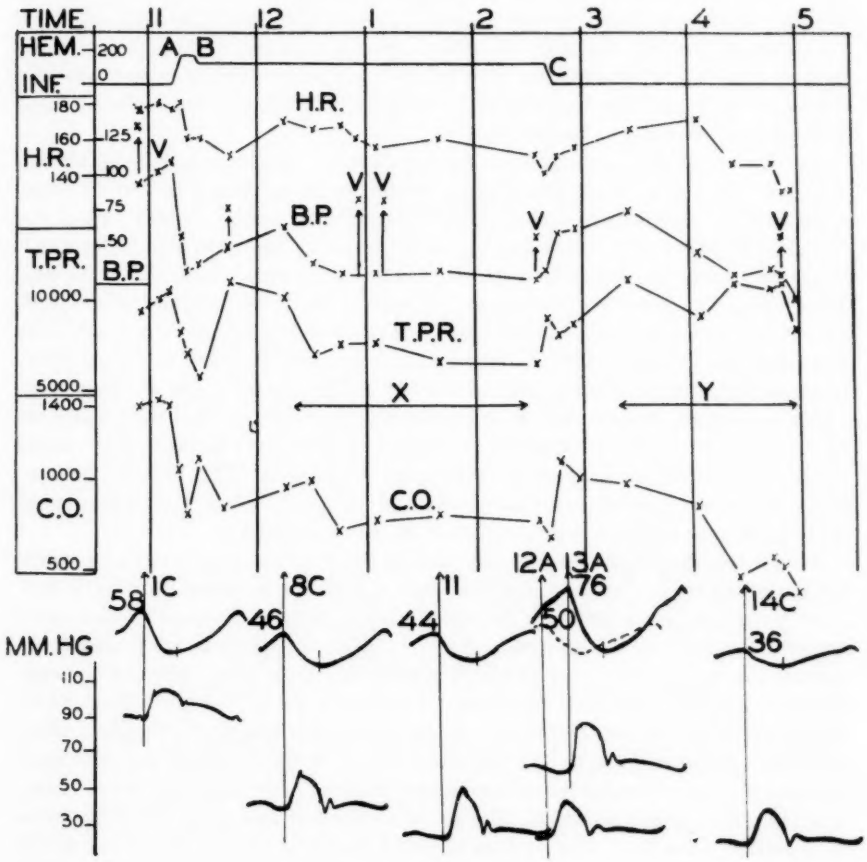


Fig. 3

two hours before reinfusion was started. The effective hypotensive period is indicated by the line X. The directional trends shown in volume curves and pressure pulses drawn below (1C, 8C, 11, 12A) are those anticipated from a decreasing venous pressure, except that the total decrease in venous pressure was not large. The plot of TPR during the post-hemorrhagic period in this experiment shows a progressive reduction which, conjointly with the reduced output (CO), is responsible for the rapid decline of arterial pressure to pre-

carious levels. Contrary to the experiment of figure 2 also, TPR increased after reinfusion of blood, but never above normal during the significant post-infusion period (Y) in which the arterial pressures and pulses deteriorate progressively.

Stimulation of the left vagus at various times (denoted by V) showed at all times a marked increase in pressure, as well as in TPR (not charted) proving that reduction of TPR during the post-hemorrhagic period was not due to the inability of the vasomotor center to react. It must, therefore, be assigned either to failure of the afferent sides of pressor arcs or to some dominant peripheral action. In two other animals, a reduced tolerance to loss of blood was also associated with decrease in TPR, which suggests a possible factor in the variable resistance of animals to hemorrhage.

In this or similar experiments any functional damage inaugurated during the phase of hemorrhagic hypotension could not have been initiated by vasoconstriction. Nevertheless, during the post-infusion period (Y), failure of the circulation progressed much more rapidly than in the previous experiment. At autopsy, the duodenal mucosa showed marked hemorrhagic changes, with presence of free blood in the lumen. A careful study of volume curves, illustrated by a few transcribed curves, again revealed no significant deviation from the mechanism described by Y. Henderson. Since TPR never increased above normal, the progressive decrease in cardiac output, clearly due to decreasing venous pressure, rapidly led to irreversible failure. It is perhaps hazardous to say that the realized reduction in cardiac output never leads to irreversible circulatory failure as long as compensatory increase in TPR occurs, but our experiments strongly suggest that absence or sudden failure of such a peripheral state from any cause is a potent factor in its precipitation.

This mode of failure was not followed in three of our experiments in which TPR remained high and venous pressures stayed above normal until the end, or even manifested a tendency to rise as cardiac output decreased and arterial pressures and pulses deteriorated rapidly. These experiments resembled those reported in a previous paper in which myocardial depression seemed to be suggested. Figure 4 illustrates this type of reaction in a dog that initially appeared highly resistant to loss of blood. As shown in the graphs, it required three successive hemorrhages (A, B, C) of 260, 110 and 90 cc. (total 4.6 per cent body weight) to reduce arterial pressure to about 50 mm. After the last hemorrhage, a hypotensive period less than 50 mm. was obtained and maintained for more than an hour (line X). The various curves of this chart show that previous to the third hemorrhage, the fall of blood pressure was due to a decrease in both cardiac output and TPR. This indicates that certainly in operated animals, a compensatory increase in TPR once induced may subsequently fail, or it may be delayed considerably. Thus, following the last hemorrhage (C), TPR increased progressively while cardiac output declined. The latter was due both to cardiac slowing and decreased stroke volume, shown by comparison of curves 2A, 6A and 8B. However, just previous to the infusion at D, the stroke volume in curve 11C diminished much more despite a slight but unmistakable increase in venous pressure, which in turn might be attributed to the concurrent cardiac slowing.

At D, infusion of blood was started and at E about 100 cc. had entered. As shown in curve 13A venous pressures rose extremely, the cardiac stroke became very large but owing to prompt reduction of peripheral resistance the arterial pressure and pulse were not immediately improved. However, as the action of the heart improved progressively and its rate increased, arterial pressures rose above normal levels and remained elevated for approximately 20 minutes.

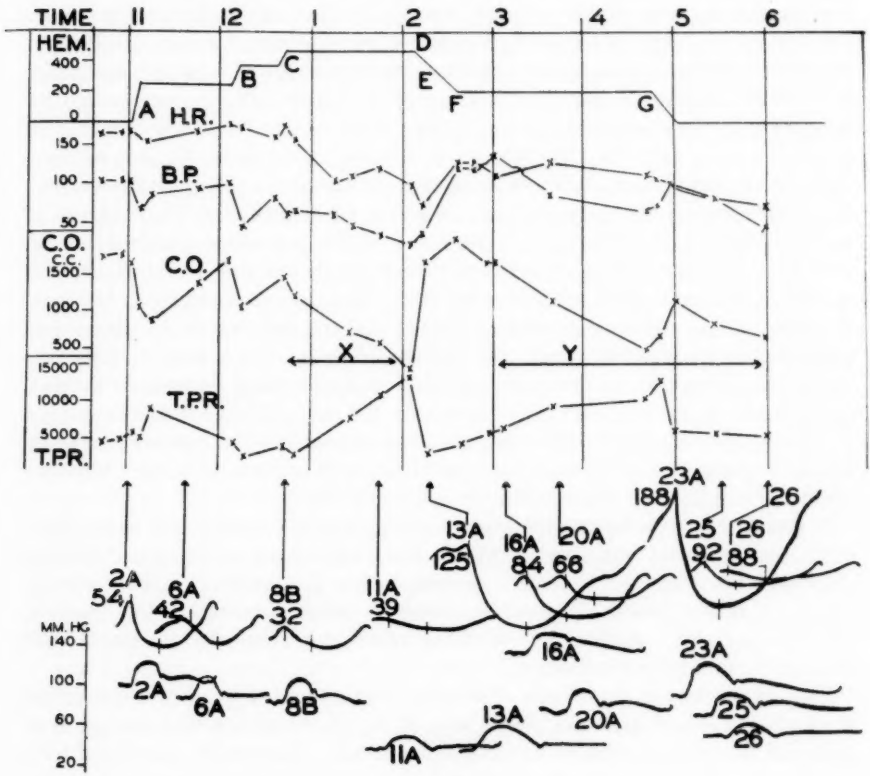


Fig. 4

After 3 p.m., however, arterial pressures and pulses began to deteriorate rapidly despite an augmenting TPR. About 4:45 the remainder of the blood still available (160 cc.) was injected. The improvement in cardiac output, arterial pressures and pulses which followed was very temporary, however, and was followed by a rapid decrease in all during the next hour. At 6 p.m. the heart became very slow and then fibrillated.

While failure to maintain a high TPR was contributory, the rapidly diminishing cardiac output was the chief mechanism that precipitated circulatory failure during the post-transfusion period (Y). But in this case it was not associated

with decline in venous pressure; on the contrary, it was attended by a significant rise until the very end. As a matter of fact, the fortuitous division of the blood for reinfusion so that a considerable volume could be given near the end made manifest a condition that might otherwise have escaped attention.

A careful survey of the volume curves confirms a suspicion already alluded to in other experiments and allows an explanation for the cardiac failure. In comparing volume curves 11A and 13A, we note the improvement in filling and the great increase in stroke volume expected as a result of the rise in venous pressures from 39 to 125 mm. H_2O which follows the first infusion (D–E); but as venous pressure subsequently decreased to 84 and 66 (curves 16A and 20A), the stroke volume reduced extraordinarily. Comparing these curves with 2A we note that the stroke volume under these pressures was actually less than at 54 mm. H_2O in 2A. The last reinfusion (G) once more raised venous pressure temporarily to 188 mm. H_2O with an enormous increase in filling rate and stroke (curve 23); but as venous pressures declined to 92 and 88 mm. H_2O (curves 25 and 26) the rate of filling became very slow and the stroke exceedingly small. This impairment of filling and ejection was clearly not due to failing venous pressures; on the contrary, it occurred with supernormal pressures. A-V constriction by the rubber dam was excluded. It was not due to an increase in hypothetical myocardial tonus, for the records as well as inspection of the ventricles in the glass cardiometer revealed that they were excessively dilated, particularly on the right side. Consequently the only allowable inference is that a hypodynamic action of the ventricles was responsible for this accumulation of blood and that the rise of intraventricular pressure thus occasioned impeded the diastolic filling (cf. curves 16A, 20A, 25 and 26).

It is true that such ventricles have not lost the power of responding to increases in venous pressures but, like the failing ventricles of a heart-lung preparation described by Starling (7), must operate under progressively higher venous pressures and progressively greater stretch in order to maintain their normal systolic discharge. Such a mode of failure certainly precipitated the irreversible circulatory failure in these dogs.

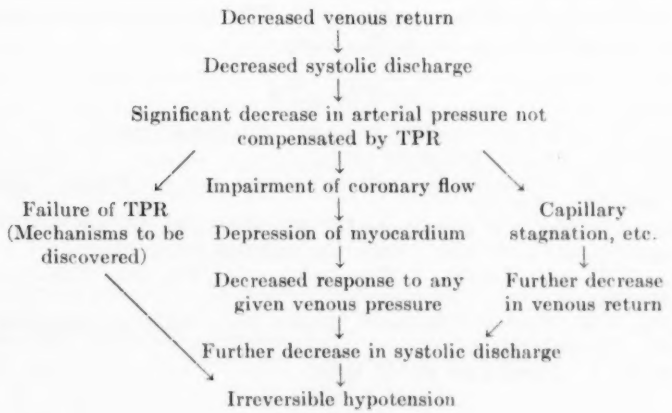
The similarities in the trends of venous pressure and arterial pressure pulses in dogs with closed and open chests suggest that such myocardial damage is at least not solely due to the more extensive operative procedures associated with the latter. Nor is it accounted for by the lowering of body temperature; many of our animals both with closed and open chests studied during the hot summer season died while temperatures were actually kept above normal. It is far more probable that the prolonged state of hypotension impairs the rate of coronary flow and while the work of the left ventricle is reduced, that of the right is probably not diminished significantly, with the result that failure of the right ventricle may precede that of the left. Admittedly, volume curves of the ventricles give no information concerning such a precedent failure of the right ventricle and our hypothesis for the present rests chiefly on observations of its dilated state through the glass cardiometer.

That such pronounced cardiac failure occurs in some dogs and not in others is readily explained when we realize that the initial condition of the myocardium

as well as the architecture of the vascular supply varies considerably in stray animals used. However, we cannot exclude the occurrence of myocardial depression even in those animals in which it is not a dominant sign. Its presence and the rôle it plays in determining irreversible failure may be concealed through concurrent reduction in venous pressures or default of the compensatory increase in TPR. When venous pressures are slightly reduced, we have no evidence as to whether the ventricles react as efficiently to the available stretching force as do the normal ventricles. The evidence from which we are accustomed to infer that the myocardium is not impaired in shock rests on repeated demonstrations that the heart is capable of responding to large infusions with increased output, but no one has demonstrated, as required according to Starling's law of the failing heart, that it responds as efficiently as a normal one to equivalent venous pressures.

In conclusion, two facts must be emphasized: 1. Our observations are limited to circulatory failure produced by a prolonged period of post-hemorrhagic hypotension and reinfusion of the withdrawn blood; they may not apply to other forms of shock. However, hemorrhage and infusion are bound to play a large rôle during warfare. 2. While our interpretations must be validated by other methods we feel justified in suggesting, on the basis of available evidence, that perhaps we have accepted too hastily the theory that the myocardium is unimpaired in shock and that the course of circulatory failure cannot be benefited by use of cardiac stimulants.

A theory of shock. Our conceptions are not in disagreement with the view that the sequence of events in shock begins with a decrease in effective circulating volume regardless of how this is accomplished. Decrease in venous return and mechanical decrease in systolic discharge as postulated by Y. Henderson and substantiated by Blalock and his associates remain early factors in initiating the sequence of events. However, unless compensatory mechanisms which increase TPR fail or unless the myocardium defaults, the precipitating mechanisms necessary to produce irreversible shock are lacking. The sequence of events which our experiments suggest may be diagrammatized somewhat as follows:


Decreased venous return
Decreased systolic discharge
Significant decrease in arterial pressure not compensated by TPR
Impairment of coronary flow
Failure of TPR (Mechanisms to be discovered)
Depression of myocardium
Capillary stagnation, etc.
Decreased response to any given venous pressure
Further decrease in venous return
Further decrease in systolic discharge
Irreversible hypotension

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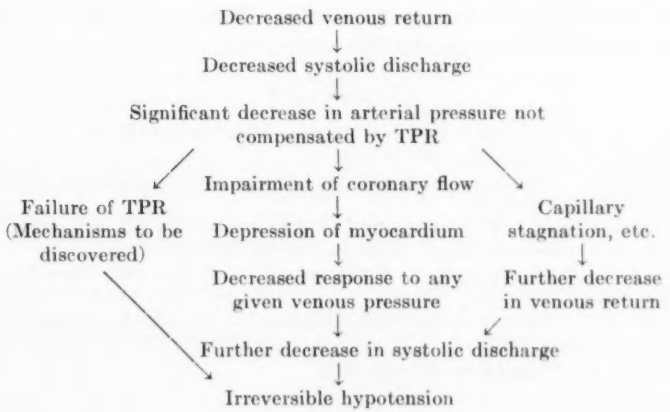
Decreased venous return
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Failure of TPR (Mechanisms to be discovered)
Depression of myocardium
Capillary stagnation, etc.
Decreased response to any given venous pressure
Further decrease in venous return
Further decrease in systolic discharge
Irreversible hypotension

SUMMARY

Dogs under morphine-barbital were bled until a marked state of hypotension was maintained for several hours. At the end of such a period the withdrawn blood (heparinized) was reinjected. A state of shock was considered to exist when such reinfusion failed to maintain arterial pressures for at least three hours and the upper intestines showed hemorrhagic changes at autopsy.

Cardiometer curves were recorded optically with aortic and venous pressures, and simultaneously on a kymograph as well. Changes in cardiac behavior were assessed from critically evaluated optical volume curves. Total peripheral resistance (TPR) was calculated from $\frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec.}}$ by use of calibrated drum records.

Experiments on 11 dogs consistently showed decreases in stroke and minute volumes during post-hemorrhagic hypotension and post-infusion failure, but the manner in which such reduction was occasioned differed. In one group, decrease in stroke was accompanied by decreasing venous pressures, in another it developed despite an elevation. Analyses of volume curves indicate that the capacity of the ventricles to respond to a given venous pressure (stretch) is reduced and that such hypodynamic action is masked when venous pressures decline concurrently. Prolonged reduction in coronary flow during severe hypotension is suggested as the cause. Our results strongly suggest that reduced capacity of the myocardium to respond to given venous pressures is one of the factors which precipitates an irreversible circulatory state.

In the majority of animals TPR increased during the period of post-hemorrhagic hypotension as well as after infusion, regardless of whether irreversible failure supervened or not. Such increase was never maximal, for stimulation of pressor nerves temporarily increased TPR tremendously. Dogs that showed little recovery of arterial pressure after initial hemorrhage, and some of those that developed rapid circulatory failure after reinfusion showed no increase in TPR. Consequently, our conclusion that development of hemorrhagic shock is not contingent on existence of high or low TPR. Persistence of an augmented TPR seems to retard rather than facilitate the development of hemorrhagic shock. Failure of a compensatory increase in TPR may be a second precipitating factor in creation of an irreversible state.

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A STUDY BY QUANTITATIVE METHODS OF THE SPONTANEOUS VARIATIONS IN VOLUME OF THE FINGER TIP, TOE TIP, AND POSTERO-SUPERIOR PORTION OF THE PINNA OF RESTING NORMAL WHITE ADULTS[1]

G. E. BURCH,[2] A. E. COHN AND C. NEUMANN

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The circulation of the blood in small blood vessels has been regarded by pathologists, physiologists and biochemists as taking an important part in the processes of inflammation, temperature regulation and the distribution and collection of food and waste materials in varying amounts during rest and activity (1, 2, 3, 4). There is information which helps to explain some of the mechanisms through which the amount of blood flowing through a tissue can be adapted to its needs. But the impression prevails that except for part-time work in carrying out these functions, the blood vessels are just small inert tubes distributing blood. That this is far from the truth is becoming evident. The small vessels contain sympathetic nerve endings through the mediation of which arterioles and capillaries react presumably upon stimulation. The rôle of the venules is not yet understood. The tissues possess local and central mechanisms whereby the amount of blood in them is varied depending upon their presumed requirements. Such variations in volume of the vascular bed are of great importance to the general circulation. They have been noticed since the very first plethysmographic studies were made, but it is only recently that systematic attempts have been directed toward analyzing them. They have been shown to depend, in part, upon the temperature of the body (5), fluctuations in tone of the sympathetic nervous system and that of local vascular beds (6) as well as the degree of reactivity of the subject (7, 8). In addition it has been demonstrated that the size of an extremity, at least of the portion studied, decreases when individuals are stimulated by pain (9).

Repeated observations, each lasting an hour or longer, have now been made of the magnitude and rhythm of fluctuations of the tips of fingers and toes and of the postero-superior portion of the pinna. In this study the term "spontaneous variations in volume" refers to those changes in the peripheral vessels which arise from intrinsic adjustments rather than as a result of stimuli deliberately applied.

MATERIALS AND METHOD. Spontaneous variations in volume of fingers, toes and ears were recorded by the pneumoplethysmographic method of Turner (10) except that cellulose acetate, instead of metal, chambers were employed. This method is sensitive and can detect changes of 0.1 cu. mm. The

[1] This is the 1st paper reporting the results of studies of the small blood vessels and related subjects.

[2] Fellow of the Commonwealth Fund.

chambers for the extremities were cylindrical (height approximately 3 cm.; radius approximately 1.2 cm.) (fig. 1 A) while that for the ear was hemicylindrical (height approximately 1.5 cm.), the radius being approximately 2.3 cm. An opening was cut to fit, without constriction, the shape of the pinna (fig. 1 B, C).

The temperature changes within the chambers, measured by constantan-copper thermocouples, were recorded simultaneously with the variations in volume. The volumes of the anatomical parts were measured by the method previously described (11). The size of the plethysmographic chambers was kept as small as possible. Although not directly utilizable in this study, changes in the effective volume of the chambers owing to the accumulation of perspired water were measured (12). Technically these measurements could not be made when anatomical variations were being studied but were under conditions as nearly comparable as possible.

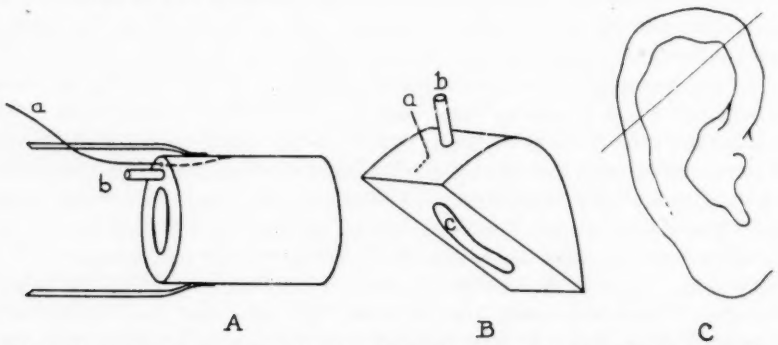

Fig. 1. Here are shown: A, the chamber for enclosing the tip of a finger or toe; B, the chamber for enclosing the postero-superior portion of the pinna (*a* represents the wires of the thermocouple; *b* is the tube leading to the recording capsule); C, the portion of the pinna lying above the oblique line which was studied.

The experiments were conducted in a medium sized air-conditioned laboratory (temperature 75°F. ± 1; relative humidity 50 per cent ± 3) which was semi-dark and sound proof. Sound within the room was limited to the almost inaudible continuous purr of air flowing from the air-conditioning unit. The room contained a hospital-type bed and the recording apparatus. It was otherwise unfurnished except for a few chairs, a large sink and a table.

Twelve normal white subjects (7 males, 5 females) varying in age from 22 to 44 years were examined. The records were made not earlier than two hours after meals. Three subjects were studied only once while the others were examined as often as two to fifteen times during the course of a year. Each subject, covered to suit his comfort, rested in bed for one hour before the chambers were placed on the tips of the right index finger and the right second toe and the postero-superior portion of the right pinna (fig. 1 C). The finger or toe tip is defined as that portion distal to a plane passing through the major distal dorsal and palmar or plantar skin creases. The parts were placed at the level

TABLE 1

Measurements of the alpha and pulse waves in 12 normal white adults

SUBJECT NO.	AGE	SEX	PART	VOLUME OF THE PART	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
					Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years			*cc.*	*cu. mm. per 5 cc. of part*		*no. per minute*			*cu. mm. per 5 cc. of part*		
Group I	39	F	F*	3.5	2.4	7.1	8.8	11	7	1.6	2.0	0.9
6			T*	4.2	2.1	7.1	6.3	10	2	1.0	1.2	0.7
			P*	2.0	3.0	5.0	9.6	12	6	1.8	2.0	1.3
33	32	M	F	5.0	4.7	18.0	9.2	12	5	2.8	3.3	2.5
			T	2.7	4.0	12.8	7.0	9	5	5.7	6.6	5.0
			P	2.7	3.7	14.7	10.0	11	7	1.1	1.5	0.9
52	44	F	F	3.2	7.1	23.3	5.8	9	4	9.8	12.4	7.5
			T	3.0	3.7	15.1	6.8	10	6	4.2	5.2	3.0
62	22	F	F	3.1	9.3	29.0	12.2	14	10	5.2	5.8	3.7
			T	3.6	5.0	12.4	9.8	10	9	3.7	4.0	3.4
82	26	F	F	4.2	4.8	12.0	8.4	10	7	3.4	4.5	2.4
			T	3.0	5.1	26.4	7.6	9	7	2.8	3.3	2.0
Mean, maximum and minimum for group I			F		5.7	29.0	8.9	14	4	4.6	12.4	2.4
			T		4.0	26.4	7.5	10	2	3.5	6.6	0.7
			P		3.3	14.7	9.8	12	6	1.5	2.0	0.9
Group II	40	M	F	6.2	12.4	32.0	7.0	8	5	5.5	7.7	3.6
67			T	4.7	8.1	25.4	9.2	13	5	3.7	4.3	3.2
			P	2.5	7.3	19.3	4.5	8	2	2.2	2.5	1.9
Group III	40	M	F	4.0	27.6	81.0	7.0	8	5	7.6	9.7	3.7
2			T	4.1	7.9	28.4	8.2	10	5	2.2	3.8	1.2
			P	1.4	7.7	18.2	10.8	12	9	1.8	2.5	1.5
3	32	M	F	4.5	20.9	53.0	6.6	9	5	9.4	11.0	5.5
			T	4.5	8.7	24.5	7.6	10	6	3.8	4.5	3.1
			P	2.3	7.0	24.0	12.4	13	10	9.9	10.5	9.2
4	27	M	F	4.8	26.0	70.3	8.4	9	7	7.0	8.4	5.2
			T	3.7	18.8	43.4	7.8	9	7	9.5	11.5	7.6
			P	2.2	11.2	21.0	3.7	5	2	6.3	6.5	6.1
17	23	F	F	3.2	21.1	59.0	9.4	10	5	8.5	9.3	7.0
			T	3.2	8.4	25.2	8.4	10	5	5.1	7.6	3.2
38	31	M	F	4.6	22.1	62.5	7.4	11	5	5.5	6.8	3.9
			T	4.3	10.7	34.5	7.8	10	5	4.6	5.5	4.3
			P	1.9	6.5	18.0	9.0	11	6	5.4	5.9	5.1

TABLE 1—*Concluded*

SUBJECT NO.	AGE	SEX	PART	VOLUME OF THE PART	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
					Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years			*cc.*	*cu. mm. per 5 cc. of part*		*no. per minute*			*cu. mm. per 5 cc. of part*		
65	39	M	F	4.1	15.7	47.0	4.5	6	2	3.9	4.1	2.9
			T	4.5	3.1	10.1	5.6	9	4	1.6	1.9	1.1
Mean, maximum and minimum for group III			F		22.7	81.0	7.2	11	2	7.0	11.0	2.9
			T		9.6	43.4	7.6	10	4	4.5	11.5	1.1
			P		8.1	24.0	9.0	13	2	6.1	10.5	1.5
Mean, maximum and minimum for all subjects			F		14.5	81.0	7.9	14	2	6.9	12.4	0.9
			T		7.1	43.4	7.7	13	2	4.0	11.5	0.7
			P		6.6	21.0	8.6	13	2	4.1	10.5	0.9

*F = right index finger tip.
T = right second toe tip.
P = postero-superior portion of right pinna.

of the subject's heart. At first each study lasted three hours, but when it was observed that for the current purpose little was gained by such prolonged observations, the period was reduced to one hour.

The values obtained have been expressed in cubic millimeters per five cubic centimeters of the anatomical part studied.[3] Five cubic centimeters was chosen because it approximates the average volumes of the parts to be studied (11, 13). All observed values have been calculated so as to be comparable; changes are therefore given as if 5 cu. mm. were the size of fingers, toes and pinnae of persons of various dimensions (table 1). Corrections were made for temperature changes within the chamber.

RESULTS. Fingers, toes and pinnae were found to undergo continuous variations in volume. The spontaneous changes which occurred ranged from less than 0.1 cu. mm. to 350 cu. mm. or more. These fluctuations fall naturally into 5 easily recognizable rhythms (figs. 2, 3).

1. *Pulse waves.* These were of course occasioned by the heart beat. Their volume varied markedly (table 1, fig. 4). It was noticed that there was a relationship[4] between the size of the pulse waves and that of the alpha waves described later. Measurements were made at 4 successive 5-minute intervals. The mean volumes were 6.9 cu. mm. in the finger tips, 4.0 cu. mm. in the toe tips, and 4.1 cu. mm. in the pinnae. The pulse waves in the finger tips were accordingly twice the size of those in the toes and the postero-superior portion of the pinnae.

2. *Respiratory waves.* The volume of the vascular beds of the three parts

[3] In this paper, changes in a part are given as cubic millimeters per 5 cc. of part.
[4] This subject will be presented in detail in another publication.

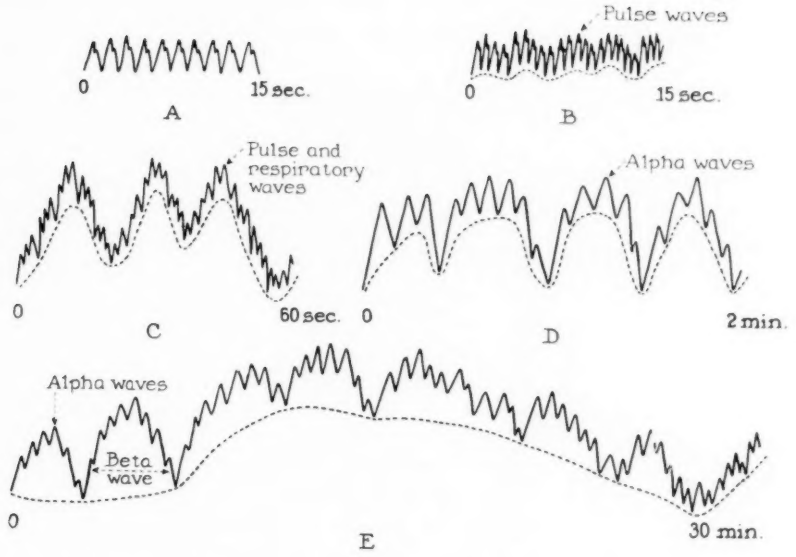

Fig. 2. Schematic drawings of 5 types of spontaneous volume waves are shown: A. *Pulse* waves are the volume changes occasioned by the heart beat. B. *Pulse* waves and *respiratory* waves. The latter are indicated by the interrupted line. C. *Pulse* waves, *respiratory* waves and *alpha* waves. The latter are indicated by the interrupted line. D. *Alpha* waves and *beta* waves. The pulse and respiratory waves are not shown. The *beta* waves are indicated by the interrupted line. E. *Alpha*, *beta* and *gamma* waves. The pulse and respiratory waves are not shown. The *gamma* waves are indicated by the interrupted line.

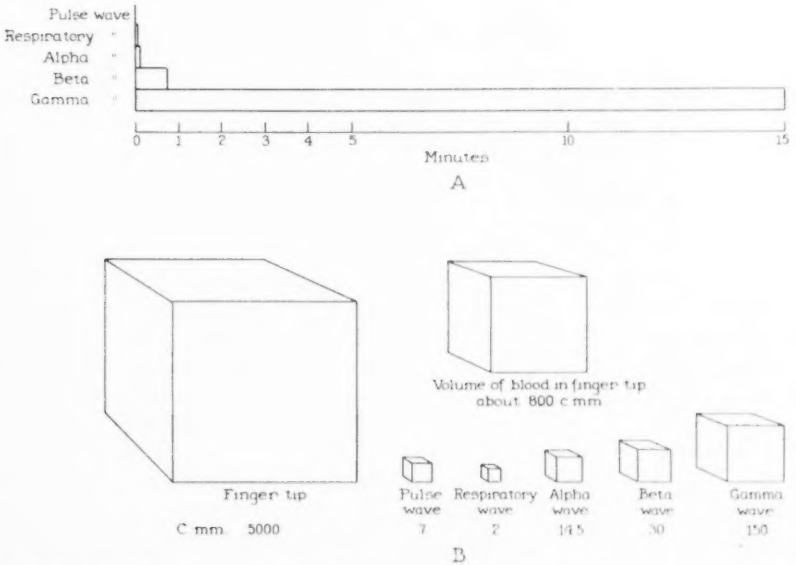

Fig. 3. A, the relative time values of 5 waves are represented by a histogram; and B, their average relative volumes by cubes of corresponding dimensions.

varied with the normal respiratory cycle. This was noticed most frequently in the pinnae but rarely in the toes. The waves occurred either in one part, in two, or in all three simultaneously (fig. 5 a). They varied from subject to subject and also from time to time in each subject, the values ranging from 0.1 to 5 cu. mm.

A special type of variation in the fingers and toes occurred within a few seconds after a deep inspiration. This form has been studied by many investigators (5, 6, 7, 14). It may exhibit several characteristics: 1. The deep inspirations responsible for the change occurred spontaneously and not as consequence of instruction or after stimulation. Shortly after them there was a sudden decrease in volume (fig. 5 B). This varied from 5 to 105 cu. mm. Associated with this the pulse wave decreased in volume. After a few pulse beats vasodilatation

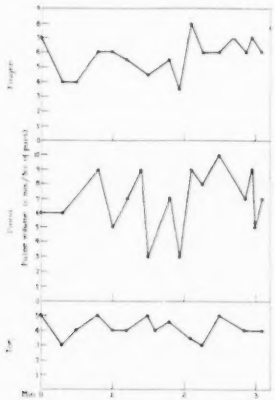

Fig. 4. These three curves show variations in volume of the pulse waves recorded simultaneously in the three anatomical parts. There are marked variations in volume of the waves in each part and discordant variations among the parts.

began and the volume gradually increased to its earlier dimension. Small variations (2 to 8 cu. mm.) similar to the alpha waves described later made their appearance just before vasodilatation was complete. Alpha waves conforming to the pattern characteristic of the subject then became reëstablished. 2. In general, the variations which depended upon deep inspiration were more prominent in the fingers than in the toes. 3. After two or more successive deep inspirations, their effect diminished until no additional response was noticed; the shorter the interval the less did the volume change. As before, the initial pattern returned. 4. The change in the pinnae was not as definite, predictable, nor as large as in the fingers and toes, nor was it necessarily concordant. There was either an increase or a decrease followed by a change in the opposite direction. These changes were similar to those described by Hertzman and Dillon (6). In one individual, a rare exception, a deep inspiration did not always produce these changes (fig. 5 c).

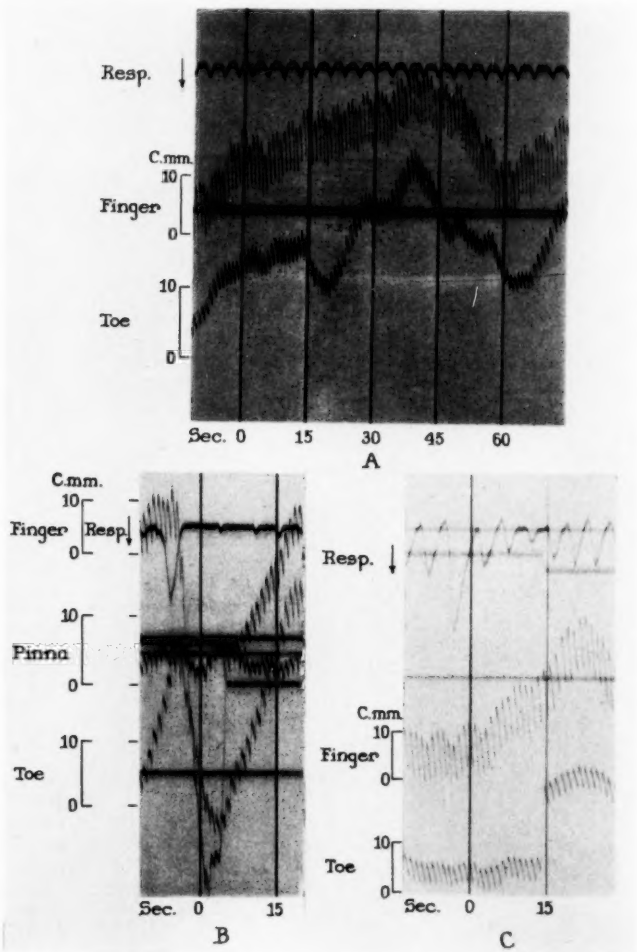

Fig. 5. The influence of respiration on volume curves is shown: A. Normal changes in the volume of the finger related to respiration. B. Sudden decrease in volume of the tips of the fingers and toes and of the pinna immediately following deep inspiration. C. Absence of vasoconstriction following deep inspiration. A downward movement of the respiratory tracings indicates inspiration. The changes are cubic millimeters per 5 cc. of part.

3. *Alpha waves.*[5] Less frequent than respiratory waves were variations to be called alpha waves. They occurred in all parts studied in all subjects. In

[5] The fact should be emphasized that as physiological realities waves do not exist, they are in this connection plethysmographic representations of complex occurrences. Upward deflection corresponds to increase in volume, downward, to decrease.

the absence of sudden changes in volume their ascending and descending limbs were of smooth contour and about equal in size; it was not at all uncommon to encounter series of unequal size. Large increases in the size of a part commonly took place slowly and usually in steps, whereas large decreases usually occurred in one long rapid deflection. In order to obtain quantitative information about

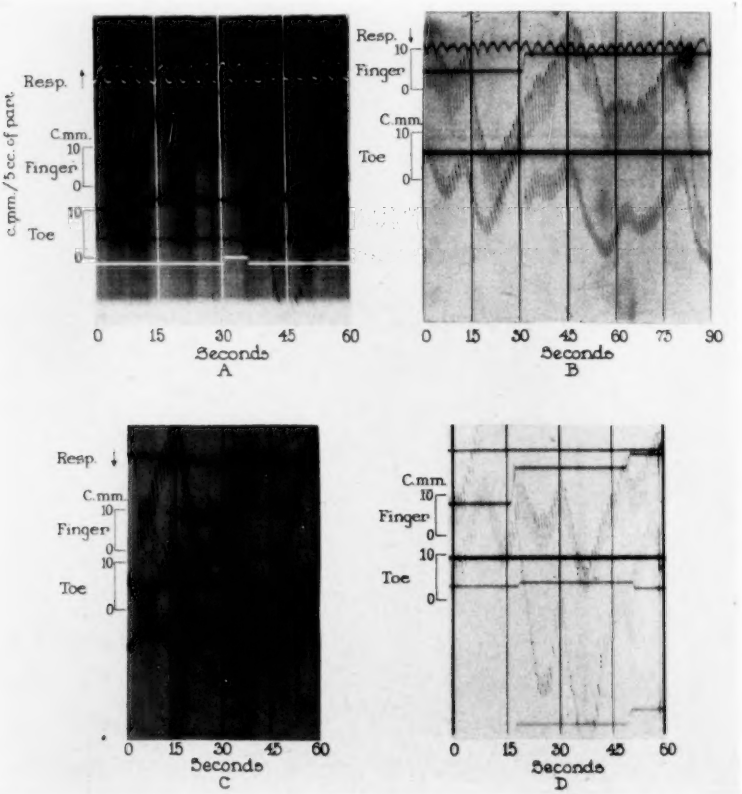

Fig. 6. Records of an individual, taken at long intervals, are closely similar, as shown in tracings of the tips of a finger and a toe at various times: A. December, 1939. B. February, 1940. C. ninety minutes after B. D. January, 1941. The volume changes are cubic millimeters per 5 cc. of part.

alpha waves, it was more practical to measure the size and frequency of their limbs rather than of the whole waves (fig. 6 A). The mean frequency of the total number of deflections (including both upward and downward) was 7.9 per minute in the fingers, 7.7 per minute in the toes, and 8.6 per minute in the pinnae. It is striking that the number observed in the three parts was not identical. The mean volume of the total number of deflections was 14.5 cu. mm. in the fingers, 7.1 cu. mm. in the toes, and 6.6 cu. mm. in the pinnae (table

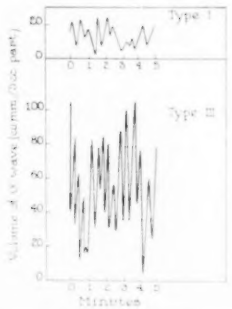

Fig. 7. Diagrams are shown to illustrate the difference between type I and type III alpha waves.

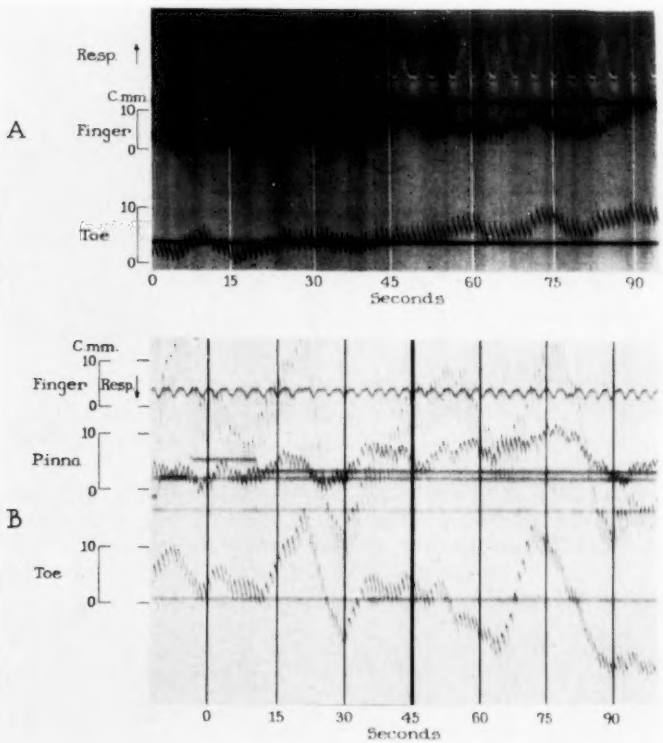

Fig. 8. Records are reproduced to illustrate typical differences: A, original tracings from a patient with type I alpha waves and B, from a patient with type III alpha waves. Inspiration is indicated by the direction of the arrows. The changes in volume are cubic millimeters per 5 cc. of part.

1). The minimum value of a single alpha deflection depends, of course, upon the sensitivity of the recording instrument. All values less than 0.5 cu. mm. were included arbitrarily in the 0.5 cu. mm. category.[6] The maximum deflection recorded was 81 cu. mm. The frequency varied from two to fourteen per minute. No correlation between frequency and volume was noticed.

The variations in the size of the limbs of alpha waves were typical of groups of

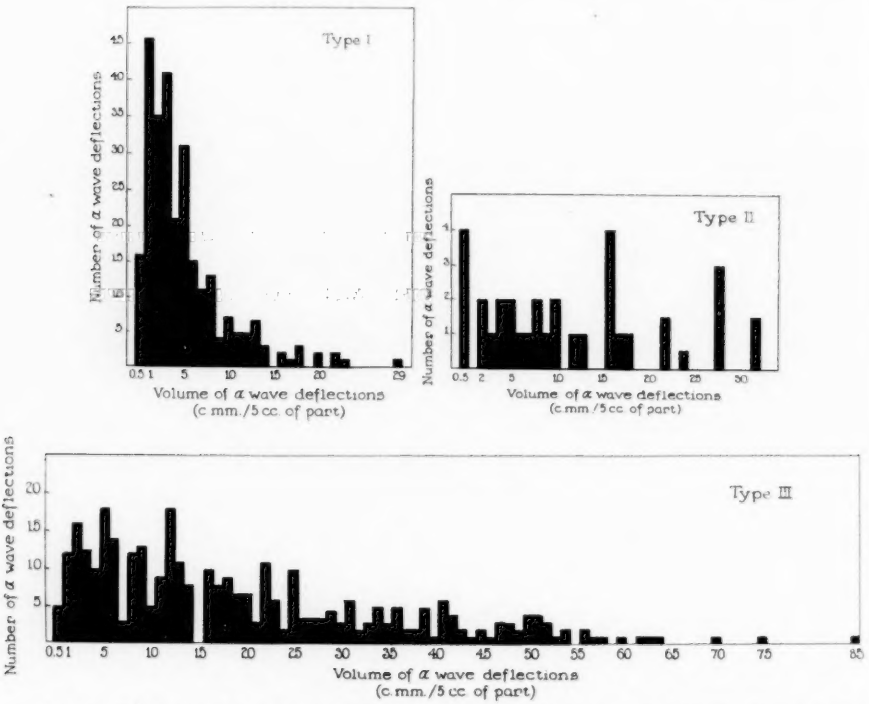
Type I
Number of α wave deflections
Volume of α wave deflections
(c mm./5 cc. of part)
Type II
Number of α wave deflections
Volume of α wave deflections
(c mm./5 cc. of part)
Type III
Number of α wave deflections
Volume of α wave deflections
(c mm./5 cc. of part)

Fig. 9. Three charts are shown to demonstrate the difference in the distribution in size of alpha waves in different groups of persons: *a*, in individuals of type I, most of the alpha waves in the finger tip during a 15 minute period in 5 subjects are small; *b*, in type II, in one subject, the sizes on the whole are larger but more scattered; *c*, in type III, in six subjects, they are also larger and much more scattered.

persons, without being characteristic of individuals (fig. 6). The curves obtained from the finger tips of twelve subjects fell into three easily identifiable types. In type I (5 subjects) almost all of the deflections represented less than 6 cu. mm. and all less than 29 cu. mm. (figs. 7, 8 and 9 A). Their sizes varied within relatively narrow limits with a mean of 5.7 cu. mm.

Characteristic of type III (6 subjects) on the other hand (fig. 9 C) was the wide range in size of the deflections, many being greater than 30 cu. mm. and a

[6] In these calculations, volumes less than 0.5 cu. mm. have no significance.

few greater than 45 cu. mm. The mean was 22.7 cu. mm. The waves of one subject, type II, were intermediate between those of type I and type III (fig. 9 B.)

The alpha waves of the toes and pinnae can in a general way be divided into three types, but with less satisfactory results than in the case of the finger tips. The alpha waves were concordant variations in all three anatomical parts for three-quarters of the time. In the other quarter the record from one or two parts showed opposite, extra, or no changes (fig. 8, table 1). Even though the changes in all the parts were unidirectional most of the time, the amount tended to be very different.

4. *Beta waves.* The succession of alpha waves was superimposed upon larger

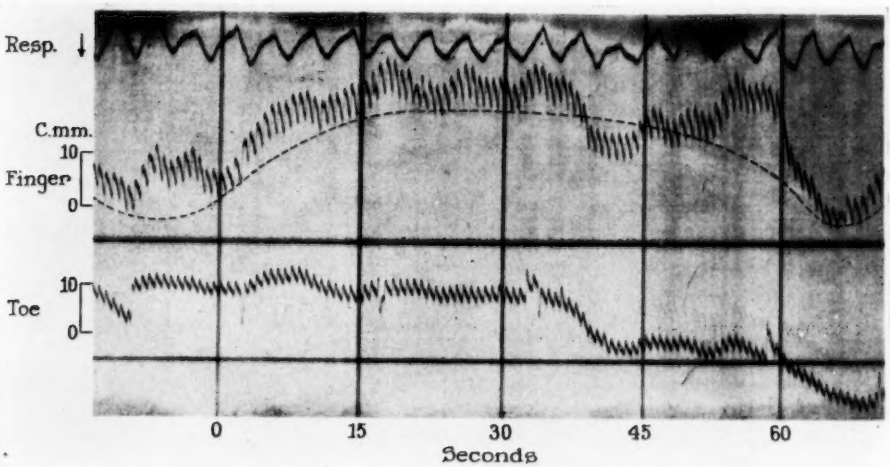

Fig. 10. A beta wave in the finger tip is indicated by the interrupted line. The duration of this beta wave is approximately 60 seconds.

waves to be called beta waves (fig. 10). The frequency of deflections was 1 to 2 per minute and the size, 5 to 60 cu. mm. All subjects and all parts studied exhibited beta waves, but there was no clearly defined general pattern as in the case of alpha waves. Their rhythm was totally irregular, but simultaneous deflections of fingers and toes and of pinnae tended to vary in the same direction.

5. *Gamma waves.* These represented slowly developing, but extensive changes in volume which were in effect the base line for the beta waves (fig. 11). The number of deflections varied between one and eight per hour and the size between 50 and 350 cu. mm. Naturally only a very small number of gamma waves were recorded in the course of the observations of any one patient, but it was evident that simultaneous gamma deflections in finger, toe and ear tended to be unidirectional.

None of the five waves described can be considered representative of an independent volume change. Each of them beginning with the pulse wave and

continuing in order through the respiratory, alpha, beta and gamma waves, is imposed in turn upon the waves next larger in size. Rhythms, the waves of which may be of greater excursion and of longer duration, may exist though they have not been detected in these studies (fig. 11).[7]

In the period during which a record was obtained, the temperature within the plethysmographic cups varied less than 1 degree and at all times changed relatively slowly. The maximum temperature change could have altered the volume of 10 cc. of air, the largest dead space in any experiment, by not more than 36 cu. mm.

During the course of each experiment there was an accumulation of perspired water. The methods used in this study precluded the possibility of estimating simultaneously the elimination of water. But the amount can be calculated, from data previously reported (12). During an hour's observation approximately 76 cu. mm. of water are secreted by the finger tip, 41 cu. mm. by the toe

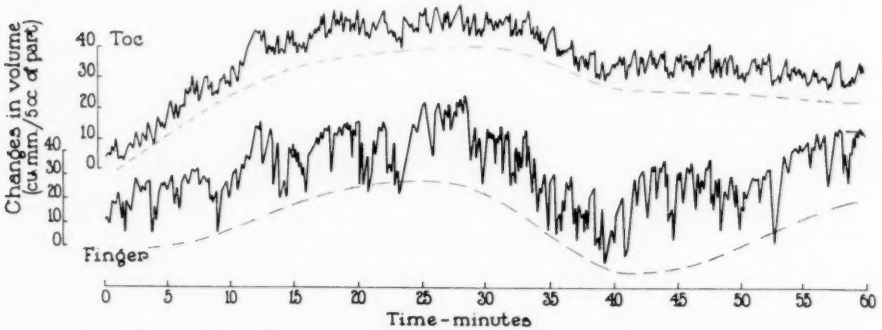

Fig. 11. The gamma waves in the tips of a finger and toe are indicated by the interrupted line. The duration of this gamma wave is 40 minutes.

tip and 25 cu. mm. by the postero-superior portion of the pinna. It appears impossible that either temperature changes or accumulations of water could modify significantly the size of any of the waves, though in an observation extending over hours the base would be slowly elevated.

Discussion. In a quantitative study of spontaneous variations in volume of certain anatomical parts it was found possible to identify and to measure the waves to which the fluctuations in volume gave rise. Arbitrary terms, alpha, beta and gamma were attached to three of the waves until a more descriptive terminology becomes available based on an understanding of their mechanism. At times beta waves were difficult to identify and seemed to represent variations in alpha waves rather than independent waves with alpha waves superimposed

[7] Of the authenticity of rapidly recurring waves there is no question. There is a question whether the waves of slow rhythms may not be artefacts owing to movements of the cups. The technique employed renders this possibility unlikely. The concordance of occurrence of waves taken from the three parts is itself evidence that physiological manifestations are being recorded.

upon them. This was possible whenever alpha waves took on dimensions approximating beta waves. In spite of such instances the beta waves were definite nevertheless, and were present in all subjects at every observation.

Although relatively prolonged, these observations were not conducted for periods of time sufficiently extended to give enough information about the gamma wave. If after three or more hours the subjects became fatigued, fatigue itself would have modified the volume changes. But in a relaxed subject five minutes of continuous recording furnished data necessary for the characterization of *alpha* waves and fifteen minutes for *beta* waves.

Although the subjects were well acquainted with the apparatus and the method used the effect of the period of rest before affixing the cups to fingers, toes and ears, was repeatedly nullified by the manipulation of applying them. Delay of another period of approximately thirty minutes was necessary before most subjects could be considered sufficiently relaxed. During this half-hour, records were made nevertheless, but the pulse waves were small and the temperatures in the chambers surrounding the parts relatively low, both events indicative of vasoconstriction. Not until vasoconstriction disappeared did the alpha, beta and gamma waves attain their full development. The measurements now presented represent periods when subjects were comfortable and relaxed. The continuous low purr of the air-conditioning unit and the comfortable atmospheric conditions contributed toward progressive relaxation. Naturally at no time was it possible to control or to be aware of what was going on in a subject's mind, but it was observed that his occasional spontaneous remarks were reflected shortly afterward by marked decreases in volume, particularly if an unpleasant thought crossed his mind such as the possibility that the apparatus might reveal the presence of a disease or that prolongation of the study would make him late for an appointment. To test this possibility the observers would ask relevant questions toward the end of each recording. Almost without fail, an unpleasant subject of discussion resulted in rapid and large decreases in volume.

In studies such as this of the external parts of the body the apparatus records the sum of a number of changes. These are related to a number of factors: 1, variations in volume of the vascular bed itself; 2, variations in volume of the air enclosed in the plethysmographic chamber owing to temperature changes; 3, accumulation of perspired water in the enclosing chamber, and 4, variations in volume of the inter- and intra-cellular fluids.

Corrections have been made for changes in temperature of the air immediately surrounding the parts. The variations in volume of the inter- and intra-cellular fluids and the relative importance of arterioles, capillaries, venules and arteriovenous communications were not ascertained. It is difficult to conceive how changes in the volume of the extravascular fluids could take place with the speed and to the extent of those recorded for each anatomical part.

The volume changes represented by the ascending and descending portions of the alpha waves fell into three types. In one (type I) they were small and the variations relatively limited. In another (type III) they were large and of

greatly variable magnitude. In one (type II) they were moderate in size, intermediate between type I and type III. The individuals with type I were placid and emotionally stable people. They were observed to adjust themselves easily to the new situation of being subjected to a physiological procedure. Some were associates in the laboratory so that it was possible to observe their reactions to the numerous and sometimes upsetting events developing in the course of a day. Subjects exhibiting type I could be depended upon to make little of disturbing environmental conditions. Those with type III reacted to their environment with wide fluctuations in mood. They were inquisitive and at times anxious about the experimental procedure. It was necessary to ask them numerous times to remain quiet, not only the first but on subsequent occasions long after they should have become accustomed to the procedure. After repeated observations no instance was found when an individual with type I waves exhibited type III waves, or vice versa.

The existence of this correlation between disposition and variations in the type of alpha waves is not surprising. Marked vasomotor changes, such as constriction or dilatation of certain blood vessels, increase or decrease in blood pressure and attacks of cardiac pain or coronary occlusion are known to be frequently precipitated by emotional strains such as fear, joy or worry. Marked changes in the volume of peripheral blood vessels are known likewise to follow pain, heat, cold, hunger and thinking. The observations now described tend to show that fundamental differences exist in the reactivity of blood vessels in types of people even when no external strain is being imposed.

The fact that there are simultaneous increases and decreases in widely separated superficial portions of the body (finger tip, toe tip and postero-superior portion of the pinna) while the circulating blood volume supposedly remains relatively constant suggests the probability of a shift back and forth of blood between superficial and internal parts of the body. Studies by others (15, 16, 17, 18, 19) have shown that in dogs there are spontaneous variations of splenic volume of a magnitude and frequency comparable to that of beta and gamma waves. The changes in the spleen as well as in other viscera may be reciprocally related to changes in the superficial portions of the body. "Spontaneous" fluctuations documented by the alpha, beta and gamma waves possibly aid in the distribution and mixing of all of the extravascular fluids and may facilitate mechanically the passage of fluid through capillary and lymphatic walls.

The mechanisms related to the alpha, beta and gamma waves are, as has been said, unknown. They are almost necessarily related to functions of the autonomic and central nervous systems and also, probably passively, to the blood pressure (5, 6, 7, 8). No data are available which associate these waves with these functions.

SUMMARY

The spontaneous variations in volume of the tip of the right index finger, the tip of the right second toe and the postero-superior portion of the right pinna of 12 normal white adults have been studied quantitatively. All parts

have undergone continuous variations in blood volume which consist of at least five separate rhythms. The effects of the heart beat and respiration were reflected in the pulse waves and respiratory waves respectively. The three other waves were arbitrarily named alpha, beta, and gamma.

The mean frequency of the alpha waves was 7.9 per minute in the finger tip, 7.7 in the toe tip, and 8.6 in the pinna. The mean volume of the deflections was 14.5 cu. mm. per 5 cc. of finger, 7.1 cu. mm. per 5 cc. of toe and 6.6 cu. mm. per 5 cc. of pinna. The frequency of the beta deflections varied from one to two per minute and the size from 5 to 60 cu. mm. per 5 cc. of tissue. The number of gamma deflections varied from one to eight per hour and the volume from 50 to 350 cu. mm. per 5 cc. of tissue.

The alpha waves obtained from the finger tips of the 12 subjects fell into three types. In type I (5 subjects) the deflections were relatively small and varied very little in size. Type III (6 subjects), on the other hand, showed a wide spread in the sizes of the deflections, many being large. Type II waves, found in a single subject, were intermediate between those of type I and type III. The subjects with type I were phlegmatic and stable while those with type III were excitable and exhibited wide fluctuations in mood.

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A STUDY OF THE RELATIONSHIP BETWEEN THE PULSE AND ALPHA WAVES OF THE TIPS OF THE FINGERS AND TOES OF FIVE ADULTS[1]

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Burton (1) has pointed out that spontaneous variations in the volume of small peripheral parts, such as fingers and toes, dependent upon the blood flow, were very large and consistently in the same direction. He stressed his belief that their essential rôle was one of temperature regulation. Hertzman and Dillon (2) have also studied such variations.

In a previous communication (3) five different kinds of waves were described documenting spontaneous variations as obtained by a plethysmographic method. Excluding the pulse and respiratory waves, the most frequently occurring ones, named alpha waves, have deflections which commonly are unidirectional in the fingers and toes and postero-superior portion of the pinna, but a more exact analysis was not made.

For more detailed study the records of five resting male subjects (4 normal, 1 senile) were selected because the usual concordant relationship, whether increase or decrease, of pulse wave to alpha wave was not uniformly observed. At the same time changes in size of alpha deflections were compared with those of pulse waves in the fingers and toes. The 5 minute period showing the most marked discrepancy was chosen for this study. Only the direction of variation in volume was used as the criterion of concordant variations in each of the four categories (fig. 1).

Simultaneous *alpha deflections and pulse waves* in the finger tips[3] were concordant in 34 per cent, and in the toe tips in 71 per cent. The range was 22 to 50 per cent in the finger tips and 62 to 76 per cent in the toe tips. In the finger and toe tips simultaneous *alpha* waves were concordant in 56 per cent, the range being 50 to 62. The *pulse* waves were concordant in 45 per cent. Forty-five per cent of the simultaneous variations in the size of the pulse waves in the finger and toe tips were concordant.

Concordant variations in size of alpha deflections and pulse waves were startlingly low in the finger tips, while the percent in the toe tips agreed in general with the previous estimates. It is not possible to assign a reason for this apparent discrepancy. In other portions of the same records, in other records of the same subjects, and in the records of other subjects it was not present. The

[1] This is the 2nd paper reporting the results of studies of the small blood vessels and related subjects.

[2] Commonwealth Fund Fellow.

[3] It was always the tips of the right index finger and of the right second toe that were examined.

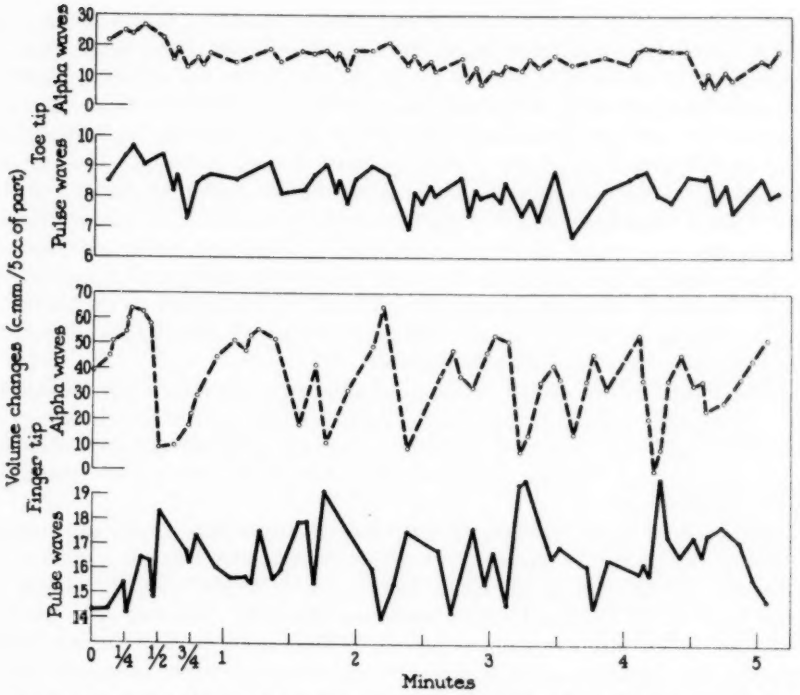

Fig. 1. Relationships are shown between the volumes of the alpha waves and pulse waves of the tips of the fingers and toes recorded simultaneously in one subject. There is marked discordance in the direction of the variations in volume of any combination of the waves of the two parts.

TABLE 1

The per cent of concordant variations in volume of the alpha and pulse waves, the alpha waves, and the pulse waves in the finger and toe tips of 5 adult male subjects

SUBJECT NO.	AGE	ALPHA WAVE		ALPHA WAVES OF FINGER AND TOE TIP	PULSE WAVES OF FINGER AND TOE TIP	DIAGNOSIS
		To pulse wave of finger tip	To pulse wave of toe tip			
	years	*per cent*	*per cent*	*per cent*	*per cent*	
2	40	36	69	62	39	Normal
3	32	22	76	50	52	Normal
35	83	33	62	59	48	Senile
38	31	50	76	52	41	Normal
90	35	31				Normal
Mean		34	71	56	45	

per cent of concordant variations of the alpha deflections and of changes in size of *pulse* waves of finger and toe tips was so close to 50 as to suggest no more than a chance relationship. In fact when the variations in *alpha* waves of the

finger of one subject were compared with those of another, the percentage of concordant changes was like that in the fingers and toes of the same subject. *Pulse* waves of two subjects told the same story. No improvement in correlation could be achieved by comparing other waves in the records of those selected. Whether this attempt should be made because an influence on the waves may occur at different times in the fingers and toes is open to doubt. Usually, in the resting, uninfluenced subject, physiological influences occur simultaneously in both. Even when the changes in any of the four categories were unidirectional, the amount was rarely the same, suggesting that the underlying mechanism, whether central or peripheral, is capable of manifesting independent activity in widely separated anatomical parts. In addition, great changes in the size of a part, as those occasioned by alpha waves, may occur with only slight and temporary changes in the size of the pulse waves.

Alpha deflections were of the usual order of magnitude and average frequency (3). Especially prominent were the marked differences in the duration of successive alpha waves as well as of those starting at nearly the same time in fingers and toes. At no time did the size of a part remain constant for more than a few pulse beats.

During a brief period of time, the *basic* size of a part may remain unchanged or it may change. If it changes, it must do so either by the accumulation or by the loss of fluid, change which can conceivably be documented by the size of alpha (and other) waves during a preceding interval. The size of a part is influenced, furthermore, during any period, by fluctuation in the volume of fluid imported into that part, again as shown by the size of the waves, especially alpha waves. A metaphor would be the influence of waves of the same size on the total configuration at high and at low tide (fig. 1). But more important than the variability of any one of the waves is the apparent lack of correlation between any two varieties as for example pulse or alpha waves. Each type of change appears to be independent. What this means in the body's economy remains obscure, but the fact that such variability can occur, even if it may be less prominent at times, would argue that local control over these waves may play a significant rôle and that vessels other than small arteries and arterioles probably exert an influence upon the size and direction of change documented by the alpha waves.

These mechanical arrangements are a background for the situations which permit appropriate accommodations in metabolism. There are involved increases and decreases in the transport of fluid in and out of anatomical parts, but also the residence of fluids in tissue spaces, in lymphatics and in other preformed locations.

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A STUDY BY QUANTITATIVE METHODS OF THE SPONTANEOUS VARIATIONS IN VOLUME OF THE TIPS OF THE FINGERS AND TOES AND POSTERO-SUPERIOR PORTION OF THE PINNA OF HYPERTENSIVE PATIENTS AND SENILE SUBJECTS[1]

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Spontaneous variations in volume of the tips of the index finger and of the second toe and of the postero-superior portion of the pinna of normal resting subjects as studied by the pneumoplethysmographic method of Turner (1) have been classified in five separate rhythms (2). The heart beat and respirations were shown to be documented in these "spontaneous" variations. The waves constituting three other rhythms could not be related to easily recognizable influences and were arbitrarily named alpha, beta and gamma.

In the present investigation a group of patients with arterial hypertension and a group of senile subjects were studied to see if there were a relation between the spontaneous variations and the nature of their ailments. The patients included 7 females with "diencephalic" hypertension varying in age from 29 to 54 years, 2 males and 4 females with "renal" hypertension varying in age from 27 to 46 years, and 7 senile males with general arteriosclerosis varying in age from 70 to 90 years.

The clinical criteria of "diencephalic" hypertension as described by Page (3) were adopted in selecting patients of this group. The essential signs are blotchy flushing of the face, neck, chest, and at times of the abdomen, watering of the eyes, excessive perspiration, palpitation and tachycardia, increase in blood pressure and coldness of the extremities. The patients with arterial hypertension of renal origin were suffering apparently as the result of chronic hemorrhagic Bright's disease or chronic pyelonephritis.

These patients were under treatment in this Hospital for periods ranging from ceveral months to years. None of the hypertensive patients was suffering from sardiac failure or more than slight impairment of renal function.

The peripheral arteries of the senile subjects were thickened and tortuous and there was arteriosclerosis of the retinal vessels. There was no diastolic hypertension. The systolic blood pressure was 165 mm. of mercury or less. They were free from congestive cardiac and renal failure. They were up and about maintaining an active daily schedule.

The method and apparatus employed in this study were the same as those used in investigating normal individuals (2).

RESULTS. The waves previously described as "spontaneous" variations

[1] This is the 8th paper reporting the results of studies of the small blood vessels and related subjects.

[2] Commonwealth Fund Fellow.

in volume of peripheral parts of normal subjects were demonstrable in all of the patients. These included the pulse, respiratory, alpha, beta, and gamma waves.

TABLE 1

Measurements of the pulse and alpha waves in patients with diencephalic hypertension

SUBJECT NO.	AGE	SEX	BLOOD PRESSURE	PART	VOLUME OF PART STUDIED	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
						Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years		*mm.* Hg		*cc.*	*cu.mm./5 cc. of part*		*No. per minute*			*cu. mm./5 cc. of part*		
20	30	F	230/134	F*	3.6	13.5	37.9	8.8	11	6	7.9	10.7	5.4
				T*	4.3	9.2	21.0	8.4	12	7	4.8	7.1	3.1
59	41	F	200/120	F	5.3	9.5	24.6	8.8	10	8	3.5	4.3	2.8
				T	3.4	5.4	14.6	7.6	9	6	1.5	1.8	1.2
				P*	2.2	2.8	6.9	14.6	15	10	5.6	6.2	4.6
70	54	F	230/120	F	3.9	10.7	34.6	7.8	9	7	5.9	7.2	4.6
				T	3.0	5.3	14.1	9.6	11	7	4.3	4.5	3.6
75	29	F	210/122	F	4.0	13.5	35.0	9.0	10	8	5.6	7.0	3.9
				T	3.9	8.4	17.8	8.0	10	7	3.7	4.9	2.2
				P	1.9	5.9	18.9	10.6	12	9	2.7	3.2	2.1
81	38	F	220/130	F	3.1	22.5	65.7	7.8	11	6	8.0	8.9	6.6
				T	3.4	15.7	67.6	7.8	10	6	5.0	9.3	1.5
				P	1.2	6.0	12.2	11.8	16	9	3.4	3.4	3.0
85	40	F	192/120	F	4.2	8.4	42.1	11.0	13	10	7.3	8.2	6.0
				T	4.0	7.1	31.3	7.6	11	5	1.6	2.2	1.3
				P	1.4	3.1	6.9	11.6	14	10	1.4	1.4	1.0
86	33	F	218/142	F	3.8	15.2	60.5	7.6	11	6	6.8	7.5	6.4
				T	3.9	7.6	36.9	8.8	11	6	2.3	3.4	1.5
				P	1.9	5.6	10.6	8.6	10	6	1.6	2.1	1.3
Mean, maximum and minimum for the group				F		13.3	65.7	8.7	13	6	6.4	10.7	2.8
				T		8.4	67.6	8.3	12	5	3.3	9.3	3.6
				P		4.7	6.9	11.4	16	6	2.8	6.2	1.0

* F = Right index finger tip.
T = Right second toe tip.
P = Postero-superior portion of the right pinna.

Pulse waves. In *diencephalic hypertension* the mean volume of the pulse wave was 6.4 cu. mm. per 5 cc. of finger tip,[3] 3.3 cu. mm. in the toe tip and 2.8 cu. mm. in the pinna (table 1). In *renal hypertension* it was 8.2 cu. mm. in the finger tip, 4.2 cu. mm. in the toe tip, and 6.5 cu. mm. in the pinna (table 2); and in

[3] All measurements of volume are in cubic millimeters per 5 cc. of part.

senility it was 6.0 cu. mm. in the finger tip, 2.0 cu. mm. in the toe tip and 4.6 cu. mm. in the pinna (table 3).

Respiratory waves. These waves were observed during quiet breathing in the three parts (fingers, toes and ears) of most of the patients. They were essentially the same as in normal individuals. There were no differences among the three groups of patients.

TABLE 2

Measurements of the pulse and alpha waves in patients with renal hypertension

SUBJECT NO.	AGE	SEX	BLOOD PRESSURE	PART	VOLUME OF PART STUDIED	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
						Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years		*mm. Hg*		*cc.*	*cu.mm./5 cc. of part*		*No. per minute*			*cu.mm./5 cc. of part*		
39	46	M	200/120	F*	4.2	14.0	38.2	7.8	10	5	12.5	15.0	7.7
				T*	4.0	7.1	22.2	11.0	13	10	5.2	6.2	4.5
				P*	2.0	5.2	17.3	10.0	17	7	9.5	9.7	9.0
40	38	F	220/148	F	3.9	19.8	54.5	11.6	12	6	6.8	7.6	6.4
				T	3.1	5.1	36.9	11.2	12	5	2.9	3.0	2.9
				P	1.2	4.5	20.0	9.8	15	6	1.6	1.6	0.8
44	42	M	198/140	F	7.1	12.3	37.3	8.0	9	6	4.0	4.6	3.5
				T	6.1	5.2	13.8	8.4	10	5	2.1	2.4	1.1
				P	3.0	4.1	10.1	10.2	14	7	8.4	10.0	0.5
57	40	F	190/138	F	3.2	12.4	47.4	7.8	10	6	5.7	7.0	4.7
72	27	F	200/140	F	3.2	14.3	50.8	14.2	18	12	9.1	10.2	6.9
				T	3.9	5.4	19.5	10.2	13	7	2.7	3.9	2.5
74	28	F	184/124	F	3.5	11.1	38.2	9.8	10	9	3.3	5.6	2.2
				T	3.3	7.7	23.0	11.2	14	10	8.3	9.7	6.8
Mean, maximum and minimum for the group				F		14.0	54.5	9.9	18	5	8.2	15.0	2.2
				T		5.1	36.9	10.4	13	5	4.2	9.7	1.1
				P		4.6	20.0	10.0	17	6	6.5	10.0	0.5

* F = Right index finger tip.
T = Right second toe tip.
P = Postero-superior portion of the right pinna.

The changes in the three parts following deep inspiration were the same as those described in normal subjects, except in the group of senile subjects in whom the decrease in volume of the tips of the fingers and toes was not as great as in the individuals with hypertension or in the normal subjects.

Alpha waves. The general configurations and characteristics of these waves in all three groups were similar to those in normal subjects. In *diencephalic hypertension* the mean volume was 13.3 cu. mm. in the finger tip, 8.4 cu. mm.

in the toe tip, and 4.6 cu. mm. in the pinna. The mean frequency was 8.7 per minute in the finger tip, 8.3 per minute in the toe tip, and 11.4 per minute in the pinna (table 1). Patients with diencephalic hypertension did not exhibit type

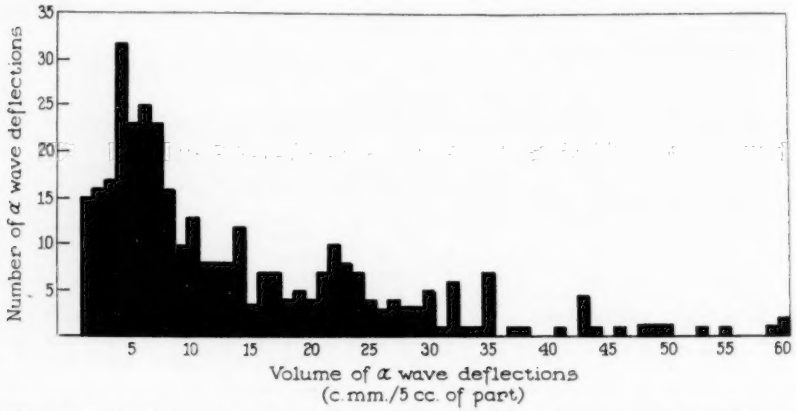

Fig. 1. The distribution is shown of the deflections of the alpha waves during a 15 minute period in 7 patients with "diencephalic" hypertension. Most of the waves are small or of medium size, but there is a wide scattering of large ones.

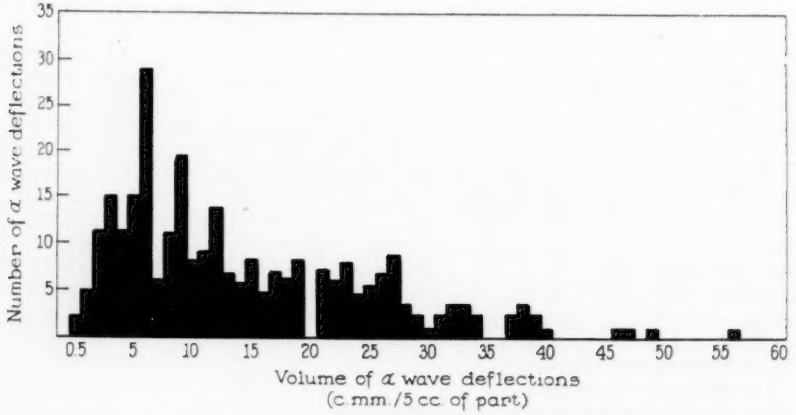

Fig. 2. The distribution is shown of the deflections of the alpha waves during a 15 minute period in 6 patients with "renal" hypertension. The sizes and spread of the waves are similar to those in patients with "diencephalic" hypertension.

I alpha waves. Five exhibited type II, and two, type III (fig. 1). In *renal hypertension* the mean volume was 14.0 cu. mm. in the finger tip, 5.1 cu. mm. in the toe tip, and 4.6 cu. mm. in the pinna. The mean frequency of deflections was 9.9 per minute in the finger tip, 10.5 in the toe tip, and 10.0 in the pinna

(table 2). The six patients with renal hypertension did not exhibit type I waves in the finger tip. Five exhibited type II, and one, type III (fig. 2). In

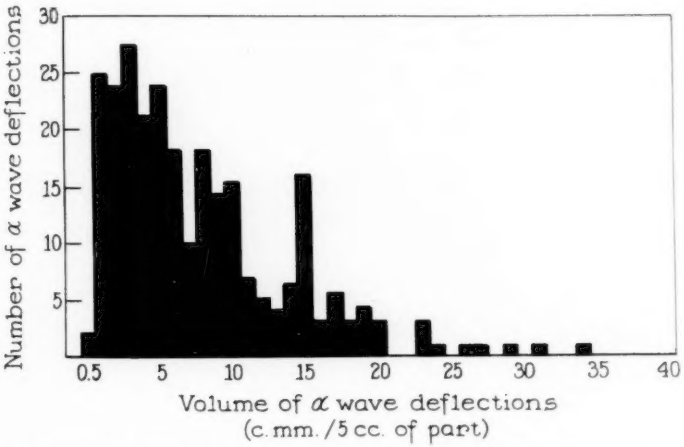

Fig. 3. The distribution is shown of the deflections of the alpha waves during a 15 minute period in 7 senile individuals. The waves are in general much smaller than those in the two groups of hypertensive patients.

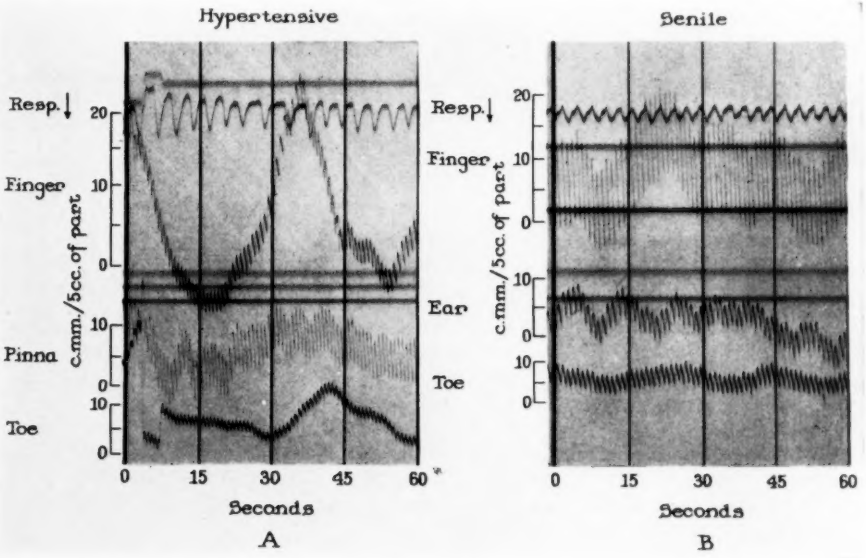

Fig. 4. Original tracings are exhibited of the alpha waves in a patient with hypertension (A) and in a senile subject (B). The waves are much larger and more variable in the patient with hypertension than in the senile one.

senility the mean volume was 8.3 cu. mm. in the finger tip, 3.5 cu. mm. in the toe tip, and 4.5 cu. mm. in the pinna. The mean frequency was 7.4 per minute in the finger tip, 5.7 in the toe tip, and 8.9 in the pinna (table 3). The alpha waves of the finger tips of all 7 senile subjects were of type I (fig. 3). Small strips of original representative tracings of alpha waves in a patient with hypertension and in a senile subject (fig. 4) illustrate the fact that the waves

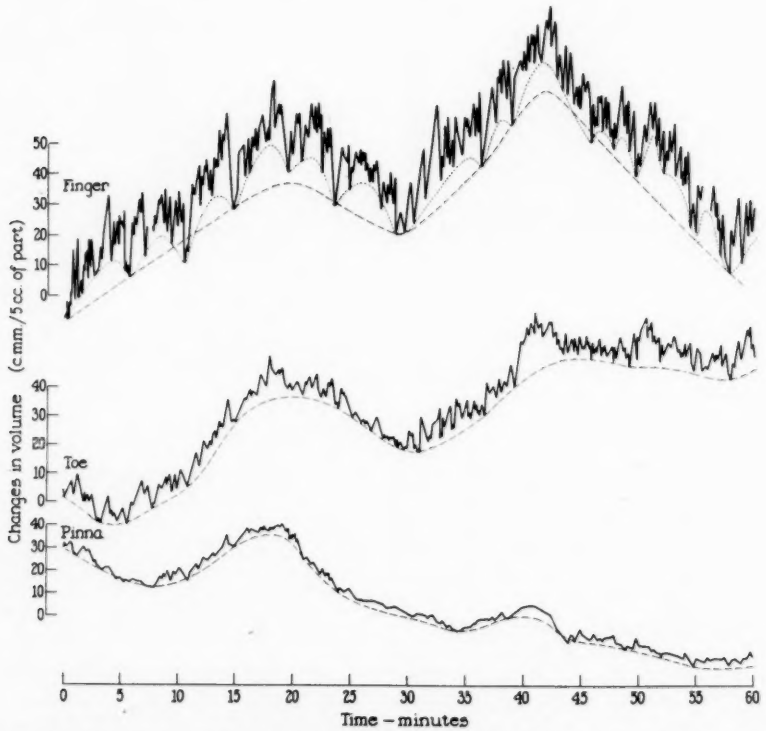

Fig. 5. Here are shown the alpha, beta, and gamma waves in the tips of the finger, the toe, and the pinna of a patient with hypertension. The gamma waves are sharply concordant in the first half. This phenomenon is less conspicuous in the last half of the tracings.

undergo greater variations in volume in patients with hypertension than in senile subjects.

Beta and gamma waves. The configurations and characteristics of these waves were essentially the same as in normal individuals. There were no significant differences among the three groups of patients (fig. 5). As in normal subjects it was not unusual for the gamma waves of one part to vary discordantly with those of the others.

DISCUSSION. The mean volumes of the pulse waves of the three groups of patients fell within a narrow range and were of the same order as those of normal subjects. One important exception occurred in the tips of the toes of the senile

TABLE 3

Measurements of the pulse and alpha waves in senile subjects

SUBJECT NO.	AGE	SEX	BLOOD PRESSURE	PART	VOLUME OF PART STUDIED	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
						Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years		*mm.* Hg		*cc.*	*cu.mm./5 cc. of part*		*No. per minute*			*cu.mm./5 cc. of part*		
27	77	M	148/82	F*	4.9	8.9	27.4	6.0	7	5	4.1	5.1	3.6
				T*	4.7	2.9	15.0	6.0	7	4	1.1	1.3	0.9
				P*	2.1	4.8	12.0	6.0	6	6	2.9	3.6	2.4
35	83	M	160/78	F	5.9	9.0	18.7	7.2	8	5	5.6	6.5	4.1
				T	4.0	3.9	17.3	7.0	9	4	1.9	2.1	1.6
				P	2.5	3.6	8.1	9.4	10	9	3.2	3.6	2.6
47	78	M	105/75	F	4.8	5.5	15.5	8.4	10	4	6.5	7.7	5.8
				T	3.5	3.0	7.0	6.2	10	4	1.3	1.6	1.1
				P	2.1	5.3	14.6	11.6	13	8	9.3	9.8	9.0
51	90	M	165/80	F	6.1	7.4	28.4	8.0	10	5	9.1	9.8	8.6
				T	4.3	3.6	10.4	4.8	6	3	1.3	1.6	0.9
				P	2.3	5.8	16.0	9.0	10	6	6.7	6.9	6.0
55	70	M	98/64	F	5.1	13.3	34.1	7.0	8	5	8.8	10.1	7.3
				T	3.7	6.3	17.8	7.6	10	5	5.5	6.6	3.4
				P	1.8	6.6	12.1	8.4	10	7	2.2	2.4	1.9
68	84	M	136/86	F	5.9	6.0	24.6	7.4	10	6	2.3	2.9	2.1
				T	3.6	2.9	8.4	3.6	6	2	1.4	2.1	1.1
				P	3.3	2.6	7.6	7.8	10	7	4.7	5.8	4.1
91	80	M	140/70	F	4.8	8.0	21.0	7.6	9	5	5.9	6.4	5.4
				T	3.2	1.6	4.8	4.8	5	3	1.4	1.6	1.3
				P	2.6	3.1	9.8	10.4	13	8	3.5	3.9	2.8
Mean, maximum and minimum for the group				F		8.3	34.1	7.4	10	4	6.0	10.1	2.1
				T		3.5	17.8	5.7	10	2	2.0	6.6	0.9
				P		4.5	16.0	8.9	13	6	4.6	9.8	1.9

*F = Right index finger tip.
T = Right second toe tip.
P = Postero-superior portion of the right pinna.

group in which the volume was one-half of that of other patients and of normal persons. The individual variations of the three parts were great in all the subjects, the maximum values being approximately 5 times the minimum. The

exception to this generalization was again the toe tips of the senile patients in which the variations were very close to the mean. The small pulse waves in the toes of the senile subjects correspond to the accepted belief that the circulation is decreased in the lower extremities of elderly individuals.

The spontaneous waves of patients with diencephalic and renal hypertension were found to be essentially the same in the resting state. Under certain circumstances of stress the peripheral blood vessels may nevertheless behave differently.

In no instance did the alpha waves of the finger tips of hypertensive patients fall into the type I classification (2). Of the 13 patients with hypertension, 10 exhibited type II waves, and three, type III. They were all emotionally unstable and excitable. They were easily disturbed by minor occurrences at home and in the hospital while under observation. They were restless, irritable and anxious during most of the time these observations were conducted. They entered a "relaxed" state with difficulty. To ascertain for what component of the total appearance hypertension on the one hand is responsible and the patient's original psychosomatic constitution on the other would require a more detailed psychometric study.

The alpha waves of the 7 senile subjects were of type I. In senile persons there was less fluctuation in the peripheral blood vessels, probably as part of their general sluggish psychosomatic activity. The extent to which this phenomenon is to be linked with the possibility that emotionally stable and phlegmatic persons live longer is a subject for further study. Marked variations in volume of the peripheral vascular bed may, of course, be inhibited by sclerotic changes. To solve such problems more extensive studies are necessary. In no patients in whom observations were repeated over a period of about one year was a change noticed in the type of their alpha waves.

SUMMARY

The spontaneous variations in volume of the peripheral blood vessels of the tip of the right index finger, the tip of the right second toe, and the posterosuperior portion of the right pinna were found to be about the same in patients with diencephalic hypertension as in those with renal hypertension. The configurations and other characteristics of the 5 types of rhythm in the blood vessels of these parts were found to be essentially the same as those described as occurring in normal subjects. In no instance were the alpha waves of these patients of type I. Of the 13 patients with hypertension, 10 exhibited type II waves, and 3, type III. All were emotionally unstable and excitable.

In senile subjects the 5 types of rhythmic spontaneous variations were also about the same as in the normal subjects and in the patients with hypertension, with the exception of the volume of the pulse waves of the toes which was smaller. Such small waves are probably due in large part to arteriosclerotic changes. The alpha waves in all of the senile subjects were of the stable variety, type I.

Whether this is due to a sluggish psychosomatic state so well known in senile individuals and also whether individuals who are emotionally stable and not easily excitable live longer is a problem which was not studied.

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THE DISAPPEARANCE RATE OF TYROSINE FROM THE BLOOD OF DOGS

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The extremely rapid disappearance rate of tyrosine from the blood stream following the intravenous administration was noted by King and Rapport (1). They were unable to find even a trace of it five minutes after the injection of 1 gram and following the injection of 5 grams there was no appreciable increase in blood amino nitrogen and only 5 to 7 per cent was accounted for by the increase in urine amino nitrogen and phenols. These observations suggested that some of the amino acid was stored in some of the body tissues. King, Simmonds and Aisner (2) found only 5 per cent of the acid, however, in the viscera and skeletal muscles 5 minutes after the injection. The content of the blood and tissues, and the urinary excretion of the amino acid and its decomposition products, therefore, accounted for only 12 to 17 per cent of the injected tyrosine. The present investigation was undertaken to ascertain whether or not a uniform disappearance rate could be obtained in normal dogs following intravenous administration of tyrosine, and to study possible factors which would alter this rate of disappearance.

METHOD. Healthy dogs were fasted 18 to 24 hours prior to the intravenous administration of 0.2 gram of tyrosine per kilogram of body weight. The tyrosine was administered as a 10 per cent solution in normal sodium hydroxide as described by King and Rapport (1). Blood phenols were measured immediately before and at intervals for 4 hours thereafter on 0.2 cc. samples of blood withdrawn from the marginal ear vein, utilizing the phenol reagent of Folin and Ciocalteau (3). There were twenty-three observations on 6 normal dogs. One dog was fasted for 7 days, and another received in addition to the daily diet, 0.2 gram of tyrosine per kilogram of weight intravenously daily for a week. The disappearance rate was then measured. Hepatic damage was produced in 1 dog by chloroform anesthesia for 2 hours on 2 successive days and the disappearance rate was ascertained on the third day. Chloroform continued to be administered to this dog until the bilirubin content of the blood serum had risen to 7.5 mgm. per cent and extreme edema had developed in the extremities, at which time the tyrosine disappearance rate was again ascertained. The disappearance rate was measured in a 10 kgm. dog 2 hours after the intravenous administration of 10 cc. of India ink. The disappearance rates from the blood and lymph were measured simultaneously in 1 dog under nembutal anesthesia and with the thoracic duct cannulated. The skin, except for that over the head and fore feet, was removed from an anesthetized dog and the disappearance of tyrosine from the blood was then measured.

RESULTS AND DISCUSSION. The tyrosine was found to disappear from the blood stream of normal dogs at a surprisingly uniform rate, although the dogs varied in weight from 10 to 20 kgm. The fasting blood tyrosine averaged from 10 to 12 mgm. per cent, reached its height of 80 to 120 mgm. per cent immediately after the injection and declined rapidly for 15 minutes to between 20 and 30 mgm. per cent. Thereafter it declined slowly, but did not reach the fasting level until after 4 hours.

When 2.4 grams of tyrosine were injected into a 12 kgm. dog whose blood volume was assumed to be 1000 cc. the maximum possible blood concentration of the amino acid would have been 240 mgm. per cent. The maximum blood tyrosine level observed was approximately 85 mgm. per cent 1 minute following the injection which required 2 minutes. Approximately 65 per cent of the tyrosine had been withdrawn from the blood stream 3 minutes after beginning of the injection. The fact that the tyrosine decreases rapidly at first from the blood stream and then very slowly for 4 hours or longer suggests that it may be temporarily stored in some tissue or tissues of the body, and then slowly liberated back into the blood stream from which it is withdrawn and metabolized. It does not appear that tyrosine is stored as such for very long. If it were, the depletion and saturation of the hypothetical tyrosine stores of the body in the 2 dogs should have altered the curves.

The tissues most likely to store tyrosine temporarily are the liver, skeletal muscles, reticulo-endothelial system and the skin. Liver damage produced by chloroform anesthesia, however, had no effect upon the rate of disappearance, although in 1 of the 2 experiments the fasting blood phenol level was 100 per cent greater than the normal. The fact that the disappearance rate was not altered following an attempt to block the reticulo-endothelial system suggests that this tissue is not one that temporarily stores the amino acid. It must be remembered, however, that filling these cells with one substance does not satisfy their appetite for another. It appears unlikely from our observation on 1 dog in which the disappearance rate was not altered following the removal of the skin from the body that the skin plays an important rôle in the temporary storage of tyrosine.

The maximum concentration of tyrosine in the thoracic duct lymph did not occur until 2 or 3 minutes after the greatest concentration in the blood and its rate of disappearance paralleled that of the blood stream. This observation suggests that tyrosine is distributed throughout the body fluids within 2 to 3 minutes after intravenous administration.

The observation of King, Simmonds and Aisner (2) that the amino acid is not stored to any great amount in the viscera or skeletal muscles and our observation that the removal of the skin did not alter the disappearance rate suggests that the tyrosine is not stored in any particular organ or tissue, but that it is distributed throughout the body fluids and cells. It appears unlikely that tyrosine is metabolized so rapidly or the urine amino nitrogen and phenols should increase more than that observed by King and Rapport (1). It is probable that the tyrosine is altered in such a manner that it is no longer detectable by procedures

commonly employed. The molecule is very likely not disrupted but it may be combined with other substances.

SUMMARY

The disappearance rate of tyrosine from the blood stream following its administration intravenously was found to be rapid and uniform in 23 observations on 6 normal dogs. The rate of disappearance was not altered by fasting, repeated injections of tyrosine, liver damage produced by chloroform poisoning, the previous injection of India ink, or by the removal of the skin. The tyrosine concentration of the lymph reached a maximum about 2 minutes after that of the blood stream and decreased at about the same rate. These observations suggest that tyrosine is distributed throughout the body and that the molecule may be altered so that it is no longer detectable by the usual procedures.

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THE ABSOLUTE THRESHOLD OF VISION IN CAT AND MAN WITH OBSERVATIONS ON ITS RELATION TO THE OPTIC CORTEX

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Experimental observations on the rat (1, 2), dog (3), cat (4), and monkey (5) have shown that surgical extirpation of the visual areas of the cerebral cortex modifies only in a minor way the ability of these animals to distinguish between lights of different intensity. The just-noticeable-difference threshold of brightness discrimination, as measured in these experiments by presenting two test stimuli of different intensity in a dark surround, was found to be slightly changed after the surgical operations were carried out. The results of these experiments have been interpreted to mean that a unitary neurological process of brightness discrimination exists which possesses only inconsequential relation to the cerebral cortex for these infra-human vertebrates. On the other hand, available data indicate that brightness discrimination in man is determined for the most part by the cortex (6, 7). Accordingly, it has been inferred that the central visual system in man is organized differently from that of lower animals, in that the function here discussed is corticalized in man, but not in infra-human vertebrates.

In order to understand more fully the nature of brightness vision in man and animals, it is necessary to determine further to what extent different aspects and conditions of brightness discrimination, other than those existing in the investigations already mentioned, are related to the functioning of the optic cortex. Some observations on this problem, already reported by one of the authors (8), have shown that in cats surgical removal of the visual cortex abolishes the ability to distinguish between differences in intensity of two test stimuli when they are presented in an illuminated surround. In order to investigate another coördinate aspect of brightness discrimination and determine whether it is dependent upon the normal functioning of the cortical optic centers, observations have been made on the absolute threshold of brightness vision for the cat and the functional value of the cortical projection areas of the retina in its determination.

The absolute brightness threshold in vision is the minimal amount of radiant energy or light which can be observed under complete dark adaptation. As such, it represents the end-point of dark adaptation. For the human eye, this value has been found to be of the order of 10^{-9} ergs (9), or as expressed in photometric units, about 10^{-7} to 10^{-6} millilamberts (10, 11, 12, 13, 14).

We have measured in photometric units the minimal amount of light at absolute threshold for the cat's eye by training the animal to press a pedal—in order to obtain food—when a large panel of flashed-opal glass behind the pedal is illuminated by white light from a tungsten lamp. If the pedal is pressed when

no light appears on the panel, an electric shock is administered. In this situation, the animal learns to press the pedal only when the light stimulus is presented.

The absolute threshold is determined under complete dark adaptation following a procedure in which the stimulus light is gradually reduced in intensity over an extended series of test trials until the ability to respond correctly fails. Thresholds have been obtained for six normal cats, on three of which the measurements were repeated. The absolute threshold of vision in these six animals varied between 5.8×10^{-8} and 2.4×10^{-7} ml., with an average value of 8.2×10^{-8} ml. Threshold values for two human subjects tested under the same conditions were 5.8×10^{-7} ml. Therefore, when measurements are made under the same conditions, the absolute threshold of the cat's eye is, on the average, about one-seventh that of the human eye.

The values obtained for the cat's eye in this experiment represent a lower absolute threshold than any of the photometric values yet reported by other investigators for the human individual. The superiority of the cat's eye over the human eye in detecting small amounts of light is probably not related to a greater absolute sensitivity of the retina of the cat. Rather, the cat's eye, by virtue of its smaller size and greater power of the dioptric system as well as the wide pupillary opening, is sufficiently "faster" than the human eye to explain the differences in the absolute threshold in the two forms. Comparison between the absolute brightness threshold of the cat's eye and that of other infra-human animals which have been tested indicates that the cat is unequalled in its ability to detect small amounts of light under dark adaptation.

Surgical operations were performed on two of these cats in which the visual areas of the cerebral cortex were partly removed in one animal (animal 1) and completely removed in the other (animal 2). In animal 1, all of the visual cortex was extirpated except for a strip of the posterior mesial surface of the gyrus splenialis on the right side. This gyrus constitutes the mesial aspect of the area striata or optic cortex of the cat. Degeneration of the external geniculate body in this animal extended throughout the nucleus except for scattered ganglion cells in the inferior medial region of the body. The optic cortex of animal 2, as well as the tissue of the gyri bordering the lateral and splenial gyri, was completely destroyed by the operation.

After recovery from the operations, the animals were again trained and tested for the absolute threshold. The results are presented in table 1, which summarizes the preoperative and postoperative thresholds obtained for these two cats. Animal 1 displayed a fivefold increase in the magnitude of the threshold after operation, animal 2 a five-hundredfold increase. The results on both animals seem to provide clear evidence that the absolute brightness threshold is directly dependent upon the normal functioning of the optic cortex. Since these findings have been obtained, confirming data have been secured by Kappauf (15) in terms of the disappearance of flicker discrimination at low brightnesses and by Mead (16) in regard to differential brightness discrimination at

low brightnesses. Both Kappauf and Mead obtained results on cats which had suffered complete removal of the visual cortex, and found that the required discriminations disappeared at a level of brightness comparable to the post-operative absolute threshold of animal 2, undoubtedly because the absolute level of sensitivity of the animals' eyes had been reached.

We conclude that the optic cortex in animals plays a significant rôle in certain aspects of brightness discrimination. It is apparently directly involved in defining the limits of vision at absolute threshold, and, as shown in a previous study cited, the cortex maintains the ability of animals to distinguish brightness differences in illuminated surrounds.

Additional information is necessary before it will be possible to suggest any general theory of the conditions under which the cortex plays an important rôle in brightness vision. But it is certain that this capacity is partly corticalized, so to speak, in the cat and in other infra-human animals, and therefore the clear-cut distinction between man and other vertebrates in this regard cannot be maintained. Since, for the cat, the functional value of the cortex in brightness vision depends on specific aspects of the stimulus conditions, the possibility arises that more thorough investigation of human individuals lacking the optic cortex might reveal some residual brightness vision mediated by subcortical centers, for it is evident that such observations as have been made on man have not been sufficiently extensive to say whether or not the visual cortex is indispensable for brightness vision.

TABLE 1

ANIMAL	TEST	THRESHOLD BRIGHTNESS	THRESHOLD INCREASED
		ml.	
1	Preoperative	1.2×10^{-7}	5 times
	Postoperative	5.8×10^{-7}	
2	Preoperative	0.58×10^{-7}	500 times
	Postoperative	0.29×10^{-4}	

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CHEMICAL COMPOSITION OF HUMAN SEMEN AND OF THE SECRETIONS OF THE PROSTATE AND SEMINAL VESICLES[1]

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The chemical nature of human semen has been incompletely described and quantitative data are lacking for important electrolytes as well as the proteins of this fluid. The seminal plasma of man is largely composed of the secretions of the prostate and the seminal vesicles. Little is known of the function of these accessory sex structures and few chemical data have been reported on the external secretions of these glands which are not known to have an endocrine secretion. The present study was conducted to determine chemical values for the principal electrolytes and proteins of normal human semen and of the secretions which are its chief constituents. The effect of varying the frequency of ejaculation on the chemistry of semen and the relationships of androgen injections were also studied.

RESULTS OF PREVIOUS INVESTIGATIONS. Chemical studies of the semen of the boar have been made by McKenzie, Miller and Baugess (1938); of the stallion by Davis and Cole (1939); of the goby (Gillichthys mirabilis) by Young and Fox (1937); and of the dog by Huggins, Masina, Eichelberger and Wharton (1939). The earlier literature on quantitative studies of human semen has been summarized by Scherstén (1936) and Zagami (1939).

Human semen. Normal human semen has a pH varying from 7.05 to 7.41 and contains large amounts of reducing substances, glucose, calcium, acid soluble phosphorus and relatively small amounts of chloride; the findings are not appreciably changed by bilateral ligation of the vas deferens (Huggins and Johnson, 1933). Goldblatt (1935) studied the phosphorus compounds and observed a high urea and lactic acid content in semen; cholesterol is somewhat lower than in blood plasma. Messer and Almquist (1937) obtained a mean value of pH 7.25 for fresh semen. Hotchkiss, Brunner and Grenley (1938) found average glucose values of 306 mgm. per cent for this fluid. Harrison (1933) obtained values for spermine phosphate, 154 to 233 mgm. for 100 cc. Scherstén (1936) reported a large content of citric acid in human semen; none was found in the seminal vesicle and the chief source of citrate was the prostate. Semen is richer than plasma in ascorbic acid (Berg, Huggins and Hodges, 1941).

Human prostatic fluid. Analysis of combined prostatic-vesicular secretions obtained by rectal massage were reported by McCarthy et al. (1928); since variable concentrations of these secretions were present in the fluids which these authors analyzed it is difficult to compare their results with the data of

[1] This investigation was aided by a grant from the National Committee on Maternal Health.

others. Huggins and Johnson (1933) found that prostatic fluid contained only small amounts of reducing substances and phosphate. Moore, Miller and McLellan (1941) obtained total cholesterol values of 314 and 332 mgm. per cent in the prostatic fluid of normal men.

Human seminal vesicle secretions. Huggins and Johnson (1933) observed high concentrations of glucose, reducing substances, acid soluble P, and non-protein nitrogen in this fluid. Berg et al. (1941) found that this structure concentrated ascorbic acid.

METHODS. Chemical studies were made on the prostatic fluid of 34 healthy men and on the seminal vesicle secretion of 7 men; these fluids were obtained by digital massage of the appropriate structure through the rectum and the first two drops of secretion appearing at the urethral meatus were discarded to minimize contamination with urine. It is difficult to obtain subjects from whom large amounts of vesicular secretion can be obtained. The semen of 56 men in good health was collected by ejaculation into a glass tube; analysis was begun after liquefaction of the semen had occurred, usually about 20 minutes after ejaculation. Microscopic examination was always done and specimens exceeding the normal count of leukocytes were discarded. The semen was well mixed by agitating the containers and for the electrolyte studies it was centrifuged at 3000 r.p.m. for 10 minutes, the analyses being performed on the supernatant seminal serum. For studies of pH and bicarbonate the fluids were delivered directly into tubes filled with oil.

Chemical methods. Water was determined by drying known weights of the fluid to constant weight; pH by the glass electrode; total CO_2 by the method of Van Slyke and Neill (1924); chloride by the method of Van Slyke (1923) as modified by Wilson and Ball (1928); sodium by the method of Butler and Tuthill (1931) as modified by Eichelberger (1938), potassium by the method of Tenery and Anderson (1940) except that pyrex tubes were used instead of quartz.

Total nitrogen and in some cases non-protein nitrogen were determined by the micro-Kjeldahl technique of Goebel (1932). Non-protein nitrogen was also determined after the proteins had been precipitated and centrifuged away, by digestion and Nesslerization of the supernatant. The proteins were estimated by multiplying by 6.25 the total nitrogen corrected for NPN. Difficulty was encountered in securing satisfactory precipitants for seminal proteins and obviously incomplete precipitation attended the use of 100 per cent ethyl alcohol, dioxane, 10 per cent trichloroacetic acid and the tungstate-sulfuric acid mixture of Folin and Wu (1919). More satisfactory results were obtained by acetone precipitation after acidification according to the method of Folin and Denis (1923); NPN was determined on the supernatant and following further washing with acetone, the precipitate was weighed. Globulins were determined by the method of Howe (1921).

The amount of protein coagulated by heat was determined as follows: a measured amount of semen in a graduated centrifuge tube was acidified to pH 5.5 with acetic acid and placed in a water bath at 100°C. for 30 minutes,

evaporation being prevented by inserting a second centrifuge tube in the mouth of that containing the sample; after centrifuging, an aliquot of the heated supernatant fluid was taken for nitrogen determination. Dialysis experiments were done by placing semen in 0.5 cc. amounts in a cellulose bag immersed in a large jar of distilled water at 37°C. Toluol was added to the sample as a preservative. The water was changed many times and dialysis was carried out for three days. As a control, blood serum was dialyzed in similar bags in each case. The amount

TABLE 1

Chemical composition of normal semen, prostatic fluid and seminal vesicle secretion

	SEMEN				PROSTATIC FLUID				SEMINAL VESICLE FLUID			
	No.	High	Low	Average	No.	High	Low	Average	No.	High	Low	Average
Values per liter of fluid												
pH	9	7.36	6.9	7.19	3	6.6	6.33	6.45	2	7.32	7.26	7.29
Water, gm.	13	944	891	918	5	936	927	932	2	900	880	890
Sodium, mM.	14	133	100	117	5	158	149	153	1	103		
Potassium, mM.	12	27.4	17	22.9	6	61.4	28.7	48.3	2	21.2	14.3	17.8
Calcium, mM.	3	7.15	5.3	6.22	3	32.7	28.7	30.2				
Total CO_2, mM.	7	33.2	19.2	24	3	5.4	3.1	4.2				
Chloride, mM.	31	57.3	28.3	42.8	8	46.1	34.8	38.1				
Acid soluble phosphorus*	8	32.3	17.2	23.8	16	1.77	0.65	1.09	7	19.8	9.65	14.7
Specific gravity	6	1039	1031	1035	14	1027	1018	1022	2	1038	1036	1037
Values per 100 cc. of fluid												
Total N, mgm.	34	1225	560	913	14	511	295	416	3	1343	1233	1284
Non protein N, mgm.	12	130	73	96	6	90	30	53.6	1			99
Total protein by difference, gm.	12	6.85	3.29	4.50	6	2.93	1.66	2.17	1			7.78
Total protein, gravimetric, gm.	11	7.74	4.30	5.80	2	2.64	2.46	2.55	1			9.04
Globulins, gm.	6	2.43	0.76	1.20								
Glucose,* mgm.	6	369	203	295	12	48	Trace	16.4	5	625	275	390
Ascorbic acid,** mgm.	9			12.8	19			0.54	9			4.66
Inorganic P,† mgm.				45								
Spermine P,† mgm.				22.5								
Urea,† mgm.				72								
Lactic acid,† mgm.				95								
Cholesterol,† mgm.				80		618	86§					

* Data of Huggins and Johnson (1933).
** Data of Berg, Huggins and Johnson (1941).
† Data of Goldblatt (1935).
§ Data of Moore, Miller and McLellan (1941).

of protein remaining in the bag was estimated from nitrogen determination following digestion of the entire bag and its contents.

RESULTS AND DISCUSSION. *Semen* was consistently slightly more acid than blood plasma and the acidity of the sample increased when ejaculation occurred at a short interval after the preceding sample was obtained. Sodium, chloride and total protein are less in amount than corresponding values of blood plasma (table 1). The content of calcium, potassium, non-protein nitrogen, acid soluble phosphorus, glucose and reducing substances including ascorbic acid are considerably increased above plasma levels.

Prostatic secretion. Compared with semen, this fluid contains more water, has a lower specific gravity and a lower pH (table 1). It also contains less chloride and total proteins while the content of glucose and bicarbonate is low. The prostatic fluid is rich in inorganic cations, notably sodium, potassium and calcium; not enough inorganic anions were found to balance the cations so as to account for the observed pH of 6.45. It is possible that the proteins being of smaller molecular size than the plasma proteins are also ionized to a greater extent and that lactate, citrate and other organic ions make up the anion deficiency. In comparing the findings in semen with those of the accessory glands it should be noted that semen is a fluid produced at least in part by nerve stimulation, while the secretion of the accessory glands was obtained during a resting period; Berg, Huggins and Hodges (1941) in the dog showed that the resting prostatic fluid obtained without adventitious stimulation differed in composition from that produced following pilocarpine injection.

TABLE 2

Dialysis of semen and serum through cellulose membranes

Time of dialysis, 72 hours

	TOTAL N	NON-PROTEIN N	TOTAL PROTEIN, ORIGINAL	TOTAL PROTEIN AFTER DIALYSIS	PROTEIN DIALYZED
			Semen		
	mgm./100 cc.	*mgm./100 cc.*	*gm./100 cc.*	*gm./100 cc.*	*per cent*
K K	676	113	3.52	1.46	58.5
S Z	780	80	4.38	1.46	66
			Serum		
K K	1118	28	6.81	6.81	0
W S	1170	38	7.08	7.06	0

Seminal vesicle secretion. When human semen stands in a glass tube following liquefaction, for many minutes the characteristic yellow translucent seminal vesicle secretion settles to the bottom of the tube and the prostatic fluid is layered above it. Vesicular secretion was found to have a high specific gravity, contained less water and was rich in proteins, NPN, acid soluble phosphorus, glucose and potassium (table 1).

The proteins of semen. Evidence was obtained from dialysis and heat coagulation that the seminal proteins are in large part derived proteins. In the dialysis experiments, 41 to 68 per cent (average 60 per cent) of the seminal proteins passed through a cellulose membrane in three days, while none of the serum proteins dialyzed simultaneously passed through this membrane (table 2).

On heating specimens of semen, either untreated or acidified with acetic acid to pH 5.5 and heated in a bath containing boiling water, only a trace of protein coagulated in four instances and 1.6 to 18 per cent of the total protein coagulated in five cases. Posner (1888) referred to the non-coagulable protein of semen as

propeptone and Goldblatt (1936) made qualitative tests which likewise supported the view that proteoses are present in semen. The present observations show that much of the seminal proteins are proteoses. It is of interest that Young and Fox (1937) observed that much of the seminal vesicle protein of the goby is secondary proteose. The globulin content of semen on average was 1.5 gram per 100 cc. and accounted for 21.4 to 40 per cent (average 29.4 per cent) of the total seminal proteins.

It was observed that gravimetric values obtained for proteins by acetone precipitation in acid solutions were consistently higher (20 to 40 per cent) than the proteins estimated by nitrogen difference (total N-NPN × 6.25). We believe that the latter method is more accurate since complex carbohydrate molecules such as mucins are known to be present in semen and precipitated by acetone.

TABLE 3

The similarity of ejaculates collected at a constant interval

S. Z. age 27 years; the interval since last ejaculation in each case is 60 hours.

DAY	CHLORIDE	TOTAL N	NON-PROTEIN N	PROTEIN
	mM/liter	*mgm./100 cc.*	*mgm./100 cc.*	*gm./100 cc.*
1	44.7	838	113	4.54
54	50.8		92.4	
61	45.6	780	92	4.22
68	44.5	820		
75	47	767	99.6	4.18
82	46.1	780	99.6	4.25
89	48.2	780	80	4.37
96	46.2	780	99.6	4.25
103	45.9	780	99.6	4.25

Factors affecting the chemical composition of semen. A similarity was observed frequently in chloride, water and protein content in samples of semen from a single individual. When semen from an individual was collected at a constant interval after the preceding ejaculation, the findings were similar over many months (table 3). Usually greater variations occurred between the semen of different individuals than between samples of one person. The factors found to modify seminal composition were great frequency of ejaculation and the androgen status.

The effect of frequency of ejaculation was studied in three men; after a control period of ejaculation at 3 to 6 day intervals semen was collected 5 times in two days. Typical results are shown in table 4; seminal samples obtained less than 24 hours after a previous ejaculation had an increased water and a decreased protein content, especially those obtained after a 6 to 14 hour interval. From the sample of 56 normal men obtained at random but known intervals, it appeared that the maximum protein content appeared between 48–72 hours after the preceding ejaculations and further continence did not result in a more con-

centrated fluid; occasionally recovery of concentration occurred between 24–48 hours.

The effect of androgen administration was studied in a man, A. M., age 26 years, who was normal except for hypogonadism due to undescended testes; prior to androgen administration this man had infantile genitalia, was beardless and while he had had numerous penile excretions there was no history of ejaculation. The androgen status of this man has been reported by Kenyon and coworkers (1937). During 36 weeks 52 nitrogen studies were made on his semen and in this period testosterone propionate, 25 mgm. daily, was injected in four courses lasting three to four weeks with intervening periods free from androgen. Semen was collected at least once each week usually 48 hours after a previous ejaculation. The maximum total nitrogen value, 1002 mgm. in 100 cc., was obtained during the course of androgen injections. There was a progressive decrease in this value each week in the androgen free periods, to 234 mgm. 84 days following cessation of testosterone injections. The chloride concentration in the periods when androgen was not injected on average was 94 millimols per

TABLE 4

The effect of frequency of ejaculation on seminal nitrogens

A. M.: age 26 years; the ejaculations are consecutive

TIME SINCE LAST EJACULATION	TOTAL N	NON-PROTEIN N	PROTEIN
hours	*mgm./100 cc.*	*mgm./100 cc.*	*gm./100 cc.*
35	975	72	5.64
40	963	72	5.57
14	555	60	3.19
10	555	60	3.19
24	848	72	4.95

liter, a figure much higher than the average for normal semen; during androgen administration chloride decreased to 53 millimols. Whenever protein increased in amount chloride decreased in the semen of this man.

SUMMARY

The proteins and principal electrolytes of semen and of prostatic and vesicular secretions were quantitatively studied in 56 normal men.

In semen, the amounts of calcium, potassium, glucose, organic phosphate and non-protein nitrogen are considerably increased above their respective concentrations in plasma; sodium and total protein and chloride concentrations are less than similar plasma values.

Human prostatic fluid has a lower specific gravity and pH (6.45) and contains more water than semen. Inorganic cations greatly exceed inorganic anions. Compared with blood plasma and semen this fluid has much less protein and bicarbonate but is richer in sodium, potassium and calcium. The seminal vesicle secretion is heavy, slightly alkaline and contains more protein than semen or prostatic fluid.

The seminal proteins are largely proteoses; on average 60 per cent of the proteins dialyzed through cellulose membranes impermeable to blood serum proteins, and only traces to 18 per cent of the proteins were coagulated in a bath of boiling water. The average globulin content of semen was 1.2 grams in 100 cc. accounting for 21.4 to 40 per cent (average 29.4 per cent) of the total seminal proteins. The protein concentration is decreased in subnormal androgen states and is increased by androgen administration. The proteins of semen are also decreased by very frequent ejaculation, the time for recovery to normal concentrations varying between 24 and 72 hours. The chemical composition is similar in repeated semen samples obtained from a single individual at a standard time since the previous ejaculation.

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HISTOCHEMICAL CHANGES IN THE MYOCARDIUM OF DOGS FOLLOWING EXPERIMENTAL TEMPORARY CORONARY ARTERIAL OCCLUSION[1]

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The effects of ischemia on muscle in general, and on the myocardium in particular, have been of continual interest to investigators of muscle physiology and pathology. The electrocardiographic and morphological changes which follow brief periods of coronary arterial occlusion have already been reported (2) (3). The present investigation is concerned primarily with the *chemical* changes in the myocardium which result from the temporary occlusion of a coronary artery.

The data have been interpreted *histochemically*. The chemical alterations have been considered as the reflections of changes in either *a*, the proportions of extracellular and intracellular tissue, or *b*, the composition of either the extra- or intracellular tissue. Other investigators have made similar morphological interpretations of chemical data in connection with other problems (4) (5) (6). Such treatment of the data seems particularly desirable in the present instance because of the great difference between the composition of the muscle fibers and of the surrounding tissue. Because of this difference, a change in the relative amounts of these two fractions of the myocardium would cause marked changes in the chemical composition of the tissue as a whole. By emphasizing the two separate fractions of the myocardium rather than the whole tissue mixture, a more intimate picture is perhaps obtained of the chemical events which follow temporary myocardial ischemia.

Chemical changes in the myocardium have been observed by previous investigators following experimental coronary occlusion. These changes consist of an increase in lactic acid and water, and a decrease in glycogen, total phosphorus and creatine (7) (8) (9) (10). Most of these observations have been made following permanent arterial occlusion or after markedly prolonged temporary occlusion. We have investigated the consequences of relatively short-term *temporary*, rather than permanent ischemia to the heart for the following reasons. Temporary relative ischemia is believed to be frequently responsible for myocardial necrosis in the human heart (3). Furthermore, as an aid toward understanding the consequences of ischemia, temporary arterial occlusion offers certain advantages over permanent occlusion. With the latter, there is little opportunity for exchange of materials between the killed or injured fibers and the rest of the body, since the affected region is shut off from the blood stream. As a consequence, substances, perhaps released by injury from the fibers, cannot

[1] A preliminary report of this study has been reported elsewhere (1).

escape from the damaged region, nor can other substances in the blood stream gain access to this region. On the other hand, in the case of a temporary occlusion, after the circulation is restored, the fibers may equilibrate via the blood stream with other parts of the body.

The difference in the results of permanent and of temporary occlusion is well illustrated in the work of Tennant *et al.* (7). These workers observed that permanent occlusion caused a marked rise in lactic acid and a fall in glycogen concentration in the cardiac muscle of the dog. However, following temporary occlusions of 2 and 8 hours, the glycogen fell as before, but no rise in lactic acid was observed. The lactic acid liberated had apparently been washed away following the restoration of the blood supply.

It was previously reported (3) that following temporary experimental occlusion of the left circumflex coronary artery in dogs, areas of necrosis developed in the myocardium, provided that the occlusion lasted longer than 20 minutes. The histological changes generally were not apparent during the first 24 hours. Electrocardiographic changes likewise resulted from the temporary arterial occlusions, but, in contrast to the histological findings, were apparent soon after the operation, and were frequently observed following occlusions of less than 20 minutes' duration.

It was considered possible that chemical examination of the myocardium following such temporary ischemia might reveal changes not recognizable histologically, and would likewise serve to supplement the histological data; to this end, the present histochemical studies were made.

The results indicate that chemical changes are present before morphological alterations are visible. One of the earliest histochemical changes resulting from temporary coronary occlusion appears to be an increase in the amount of extracellular fluid.

MATERIAL AND METHODS. *Operative procedure.* Occlusion of the left circumflex coronary artery was performed on 10 adult dogs (under nembutal anesthesia). The operative procedure has been described in detail in a previous communication (3). The artery was dissected free and occluded for 45 minutes or less by applying tension on a ligature passed beneath it. The approximate point of arterial occlusion and the myocardial area thereby deprived of its blood supply may be seen in figure 1.

In 6 control animals, identical operations were performed except that the ligature under the coronary artery remained slack, thereby causing no interference with the blood flow. The purpose of making sham occlusions was to determine whether the operation itself or the manipulation of the coronary artery, prior to the occlusion, might have had any effects upon the myocardium. These hearts also served to show the amount of variation in the chemical composition between different regions of individual hearts.

When the desired time had elapsed after the operation, the animals were again anesthetized with nembutal, and the hearts were removed while still beating.

In both control and experimental animals, samples of tissue were taken not

only from parts of the heart supplied by the left circumflex artery, but also from other regions. Thus, by obtaining tissue from control regions of the same hearts, the effects of individual variations between different hearts were eliminated.

Six samples of cardiac muscle were taken from each heart. Three "affected" samples were selected from regions of the left ventricle, right ventricle, and septum supplied by the artery that had been occluded, and three control samples were removed from regions supplied by intact arteries.

The tissue samples for chemical and histological examination were taken as soon as the beating heart was removed. Samples for electrolyte analyses were stored in stoppered weighing bottles in the ice box; samples for glycogen analysis were weighed quickly on removal and placed immediately in Zenker's solu-

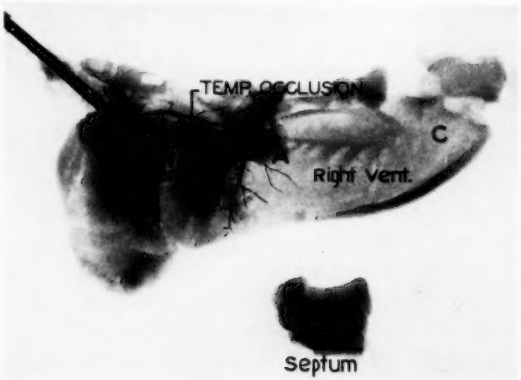

Fig. 1. X-ray photograph of the opened heart with injected left circumflex artery showing the point of temporary occlusion and the regions affected (A) and unaffected (C) which were taken for study.

tion. The speed of handling was designed to minimize loss of water or glycogen from the tissues.

Blood samples were obtained from the superior vena cava. Most of the blood taken was allowed to clot under oil, and the serum separated. One sample of blood was oxalated and preserved as a standard for calculating the amount of blood in the cardiac muscle by quantitative spectrophotometric analyses.

The serum was analyzed for water, chloride, and sodium. The cardiac muscle samples were analyzed in triplicate for chloride, in duplicate for sodium and potassium, and single determinations were made for water, fat, and blood. In some cases, glycogen was also measured.

Serum analyses. The serum chloride was measured essentially according to the Wilson and Ball modification of the Van Slyke procedure (11). The serum sodium was measured as described by Hald (12), except that a centrifuge technique was employed as outlined below for tissue. The serum water content was determined by drying 1 cc. portions at 100°C. overnight.

Tissue analyses. As soon as the samples of myocardium were chilled to the

ice-box temperature, they were trimmed free of epicardial fat and the tag ends of the chordae tendineae. By working in a cold-room, water loss during the trimming operation was kept to a minimum. The individual tissue samples were reduced to a finely divided state, suitable for the taking of aliquots, by grinding in a mortar with liquid air. Samples were then weighed for subsequent analyses.

Chloride analyses were performed on 0.3 to 0.5 gram samples as described by Hastings and Eichelberger (6).

Sodium and *potassium* were determined on a 10 per cent trichloracetic acid extract of a single 1 to 5 gram sample of tissue. Aliquots of this trichloracetic acid extract were evaporated with 0.5 cc. of 10 N sulfuric acid per gram of tissue, and wet ashed in pyrex tubes with the repeated addition of small amounts of nitric acid. Following the wet ashing, phosphate was removed by adjusting the pH to 5.0 with redistilled ammonium hydroxide.

Aliquots of the phosphate-free solution were heated in platinum crucibles to 500–550°C. in a muffle furnace, and the potassium in the ash was determined gravimetrically as the chloroplatinate. The conditions employed for precipitation of the potassium chloroplatinate were approximately those described by Hald (12). However, the precipitate was isolated by centrifuging and washing in a tube with a pointed tip, rather than by filtration.

Sodium was determined on the supernatant fluid and washings which were siphoned from the tube containing the potassium chloroplatinate precipitate into another (5 cc.) centrifuge tube. These alcoholic washings, containing the sodium, were evaporated by placing the centrifuge tube in a water-bath at 50° and directing a jet of air into the tube. When not quite dry, the tube was cooled and 4 cc. of Barber-Koltoff uranyl-zinc-acetate reagent was added. Thorough stirring was accomplished by twirling in the tube a slender glass rod provided with a sine-wave-shaped bend a few centimeters above the lower end. After standing 30 minutes, the tube was centrifuged, and the supernatant fluid siphoned off. Washing of the precipitate was accomplished by stirring up the precipitate with 4 cc. of a wash solution and recentrifuging. The precipitate was washed twice, once with each of two different washes both of which had been saturated with sodium zinc uranyl acetate. The first wash consisted of 78 volumes of acetic acid, 20 volumes of 95 per cent alcohol, and 2 volumes of Koltoff reagent. The second wash contained 95 volumes of 95 per cent alcohol, and 5 volumes of acetic acid. During hot, humid weather, these operations were carried out in a cold-room. After drying the tubes overnight at 38°C., they were weighed on a microbalance. Duplicate analyses checked within 1 or 2 per cent, and known solutions gave values within 1 per cent of the actual content.[2]

[2] A slight improvement was later made in these washes. The first wash was changed to glacial acetic acid plus 2 per cent by volume of the Koltoff reagent; and the second wash consisted of 10 volumes of acetic acid, 80 volumes of 95 per cent alcohol, and 10 volumes of the first wash, both washes being saturated with triple salt just before use. The second wash was prepared freshly each time it was used. To insure the removal of the last traces of solvent, it was found expedient to heat the tubes for 1 hour at 100° before weighing.

Fat and *water* concentrations were determined as described by Hastings and Eichelberger (6). The residual *blood* in the tissue was estimated either by comparison of the animal's own blood with an aqueous tissue extract after conversion to acid hematin (6), or by measurement of the hemoglobin itself in blood and tissue by the use of the spectrophotometer. The difference in the position of the myoglobin and hemoglobin absorption bands permitted correction of these figures for myoglobin in a manner suggested by Watson (13).

The *glycogen* measurements were performed according to Good, Kramer, and Somogyi (14), with slight modifications.

Calculations. All tissue data were calculated on a blood-free, fat-free basis. From the sodium and chloride values the amount of the extracellular fluid was calculated as detailed by Hastings and Eichelberger (6). The calculations were somewhat modified through the use of the assumption that the extracellular fluid of the heart muscle contains 4 per cent protein instead of none as had been assumed in the case of skeletal muscle. The assumption of this concentration of protein in the extracellular fluid is in keeping with the observation of Drinker *et al.* (15) on the composition of cardiac lymph. As a consequence, the Gibbs-Donnan ratio for the distribution of ions between serum and extracellular fluid was estimated as 0.98 instead of 0.95. The grams of total extracellular tissue per kilogram, E, was estimated as the sum of the extracellular fluid and the collagen, which was assumed to be 10 grams per kilogram in the muscle of the left ventricle and septum, and 14 grams per kilogram in the muscle of the right ventricle (16).

The symbols E_{Cl} and E_{Na}, are used to signify whether the amount of extracellular tissue per kilogram of muscle was calculated from the chloride or from the sodium concentrations, in one case the chloride and in the other case the sodium being assumed to occupy an exclusively extracellular position. The relative mass of the intracellular compartment, C, was determined by subtracting the average of E_{Cl} and E_{Na} from 1000.

The concentration of water and potassium in the fibers, $(H_2O)_C$, and $(K)_C$, was likewise calculated, after deducting the extracellular water and potassium.

These histochemical calculations have been made not only for the control samples of cardiac muscle, but also for the samples that were removed from the regions of the heart that had suffered a period of ischemia. It is, of course, possible that as a consequence of damage to the fibers, chloride or sodium or both might have entered the muscle fibers, in which case these ions would no longer serve as accurate measures of the amount of extracellular fluid. Fortunately, the results of the calculations themselves give some evidence as to the validity of the assumptions used in making the calculations. This point will be discussed together with the data.

RESULTS. The data are presented as *a*, the original analytical values for water, chloride, sodium, potassium, and glycogen; and *b*, the derived histochemical values obtained from these primary data.

For each part of the myocardium—i.e., left ventricle, septum, and right ventricle,—values are given side by side in the tables for both the region mainly

supplied by the artery occluded and for a region not directly supplied by this artery. These regions are called "affected" and "control" respectively. The completeness with which the affected portion was supplied by the occluded artery is undoubtedly different in each of the 3 parts of the myocardium, and

TABLE 1

Analysis of control hearts

$(H_2O)_c$, $(K)_c$, $(Gly)_c$ based on a kilogram of fibers.
Other data based on a kilogram of fat-free, blood-free tissue.

	TISSUE	H_2O	Cl	Na	K	GLYCOGEN	E_{Cl}	E_{Na}	C	$(H_2O)_c$	$(K)_c$	$(Gly)_c$
		grams	*mEq.*	*mEq.*	*mEq.*	*grams*	*grams*	*grams*	*grams*	*grams*	*mEq.*	*gram*
	Serum	921.9	114.8	143.0								
	L.V. Control	780.7	24.0	30.0	94.1	9.4	209	217	787	748	118	11.9
	L.V. Affected	779.0	23.2	28.2	91.2	9.3	202	205	796	748	114	11.7
Dog 1, 4.5 hours post operatively	Change	−1.7	−0.8	−1.8	−2.9	−0.1	−7	−12	+9	0	−4	−0.2
	Sep. Control	778.9	22.4	30.3	92.7		195	219	793	746	117	
	Sep. Affected	777.8	23.7	32.6	88.9		206	235	780	742	113	
	Change	−1.1	+1.3	+2.3	−3.8		+11	+16	−13	−4	−4	
	R.V. Control	791.3	25.5	33.8	90.6		225	248	764	760	117	
	R.V. Affected	788.0	25.7	33.1	93.6		227	243	765	758	121	
	Change	−3.3	+0.2	−0.7	+3.0		+2	−5	+1	−2	+4	
	Serum	925.2	111.9	139.2								
	L.V. Control	783.3	24.0	30.9	85.2	9.8	219	231	775	748	111	126
	L.V. Affected	782.5	24.5	32.0	90.6	10.0	223	239	769	745	116	132
	Change	−0.8	+0.5	+1.1	+5.4	+0.2	+4	+8	−6	−3	+5	+6
Average of 6 controls, 4-288 hours post operatively	Sep. Control	782.6	23.3	31.8	90.6		213	232	776	746	116	
	Sep. Affected	779.9	23.8	32.7	88.5		217	238	771	744	113	
	Change	−2.7	+0.5	+0.9	−2.1		+4	+6	−5	−2	−3	
	R.V. Control	788.5	24.9	32.6	92.8		230	242	765	755	120	
	R.V. Affected	786.0	25.1	32.4	91.0		233	240	764	754	118	
	Change	−2.5	+0.2	−0.2	−1.8		+3	−2	−1	−1	−2	
	σ*	6.5	1.7	2.5	5.4		15	23	17	6	6	
	σ†	1.5	0.5	1.2	2.3		5	9	6	2	3	

* Comparison with homologous regions of all 6 hearts.
† Two parts of same region of same heart compared.

in fact, in the same part of different hearts. As a result, the degree of ischemia produced by temporary occlusion would be expected to differ in the 3 divisions of the heart, and also in different hearts.

Analyses of control hearts. In table 1 are shown the results of 6 control experiments in which a mock occlusion of a coronary artery was performed and the tissue removed 4 to 288 hours later, 4 of the 6 being removed from 4 to 29

hours after operation. "Affected" signifies the region that would have been affected had the blood flow actually been interrupted by the ligature. One typical experiment is recorded completely; and in addition, the average for all the experiments is given together with the change between "affected" and "control" areas.

The average results fail to indicate any significant difference between "control" and "affected" portions, and it seems permissible to conclude that in the absence of actual occlusion the operative procedure employed did not materially affect any given region of the heart. No abnormal histological changes were evident in any of these hearts.

The data for these control experiments may, therefore, be examined to see what variations occur between different regions of the same heart or between the same regions of different hearts. As a measure of these variations, the standard deviations have been recorded in two different ways. First, there is given the standard deviation of individual values from the average value for all of the tissues from homologous regions of the 6 hearts—e.g., individual left ventricle values are compared with the average of all left ventricle values. Second, there is given the standard deviation of individual values from the average of the two values, "control" and "affected," for the same part of the same heart; —e.g., an individual value for the left ventricle "affected" is compared with the average of the left ventricle "affected" and "control" in the same heart.

As might have been anticipated, the correlation is much better between nearby regions of the same heart than between homologous regions of different hearts. Therefore, in the case of actual occlusions, there appears to be a real advantage in obtaining control samples from the same heart, and from each of the ventricles and the septum of that heart.

On the average, there is good correlation between the relative amount of extracellular tissue calculated from chloride, E_{Cl}, and that calculated from sodium, E_{Na}. However, the extracellular values estimated from the sodium data averaged 7 per cent greater than those calculated from the chloride figures, which suggests the presence of a small amount of intracellular sodium, perhaps 3 milliequivalents per kilogram of fibers. In spite of the considerable variation in the amount of extracellular fluid in different hearts, the water content of the fibers, $(H_2O)_C$, showed only moderate fluctuations.

The relative amount of extracellular tissue in the control hearts varied from 200 to 260 grams per kilogram of blood-free, fat-free tissue. Slightly higher values were found in the right ventricle than in the septum or left ventricle. This difference, although small, is consistent with the work of previous investigators who found higher chloride and water concentrations in the right ventricle than in the left (17) (18). The right ventricle thus appears to be a little more loosely constructed than the rest of the myocardium.

Analyses of hearts removed 4 to 5 hours after temporary arterial occlusions. In table 2 are recorded the quantitative chemical changes present in the hearts of 4 dogs, 4 or 5 hours after the termination of temporary occlusion of a coronary artery. The actual occlusions lasted 15 minutes in one case, and 45 minutes in the rest. The results in 2 typical cases and the average of all 4 are presented.

In general, there is an increase in the water, chloride, and sodium concentrations in the myocardium with no conclusive change in potassium or glycogen.

TABLE 2

Analyses of hearts removed 4 to 5 hours following temporary arterial occlusions

$(H_2O)_c$, $(K)_c$, and $(Gly)_c$ calculated per kilogram of fibers.
Other data based on a kilogram of fat-free, blood-free tissue.

			ORIGINAL DATA					DERIVED DATA					
			H_2O	Cl	Na	K	Glycogen	E_{Cl}	E_{Na}	C	$(H_2O)_c$	$(K)_c$	$(Gly)_c$
			grams	*mEq.*	*mEq.*	*mEq.*	*grams*	*grams*	*grams*	*grams*	*grams*	*mEq.*	*grams*
	L.V.	Control	780.4	19.8	24.7	87.8	6.5	195	190	808	750	107	8.1
	L.V.	Affected	786.3	24.8	28.8	82.4	7.6	241	219	770	748	105	9.9
		Change	+5.9	+5.0	+4.1	−5.4	+1.1	+46	+29	−38	−2	−2	+1.8
No. 2*, 45 minutes occlusion; 4.5 hours after occlusion	Sep.	Control	777.9	20.8	24.5	86.0		204	188	804	748	105	
	Sep.	Affected	787.0	23.6	28.9	89.2		230	220	775	752	114	
		Change	+9.1	+2.8	+4.4	+3.2		+26	+32	−29	+4	+9	
	R.V.	Control	772.9	19.5	24.2	87.9		196	190	807	748	108	
	R.V.	Affected	779.1	20.7	25.1	90.1		207	197	798	752	111	
		Change	+6.2	+1.2	+0.9	+2.2		+11	+7	−9	+4	+3	
	L.V.	Control	781.3	27.0	32.4	88.8	6.3	247	243	755	739	116	8.3
	L.V.	Affected	781.0	29.7	33.4	88.9	6.7	270	251	740	734	115	9.1
		Change	−0.3	+2.7	+1.0	+0.1	+0.4	+23	+8	−15	−5	−1	+0.8
No. 3, 45 minutes occlusion; 4 hours after occlusion	Sep.	Control	779.5	25.9	31.4	89.4		237	236	764	738	116	
	Sep.	Affected	806.2	46.2	59.8	76.2		412	438	575	712	130	
		Change	+26.7	+20.3	+28.4	−13.2		+175	+202	−189	−26	+14	
	R.V.	Control	781.0	28.0	34.4	91.4		259	261	740	739	122	
	R.V.	Affected	786.2	30.1	35.9	94.3		279	273	724	743	128	
		Change	+5.2	+2.1	+1.5	+2.9		+20	+12	−16	+4	+6	
	L.V.	Control	785.5	23.8	29.3	88.2	6.6	214	218	785	752	111	8.4
	L.V.	Affected	787.0	28.8	32.2	87.3	7.0	241	237	761	748	112	9.4
		Change	+1.5	+5.0	+2.9	−0.5	+0.4	+27	+19	−24	−4	+1	+1.0
Summary of 4 hearts removed 4–5 hours after occlusion	Sep.	Control	783.5	23.2	29.0	91.5		210	214	789	747	115	
	Sep.	Affected	793.8	34.0	38.4	88.2		260	281	736	733	121	
		Change	+10.3	+10.8	+9.4	−3.3		+50	+67	−53	−14	+6	
	R.V.	Control	782.7	24.1	30.7	95.1		224	233	773	746	123	
	R.V.	Affected	785.4	26.4	32.8	93.6		245	245	755	744	123	
		Change	+2.7	+2.3	+1.1	−1.5		+21	+12	−18	−2	0	

* The dogs numbered 1, 2, 3, 4, 5 in this paper correspond with those numbered 71, 70, 69, 54, 55, respectively, in the paper (3) in which the electrocardiographic and morphological studies have already been reported in detail.

The failure to observe the fall in glycogen that others have found may be due to the relatively short duration of these occlusions. The different divisions of the heart evidence different degrees of change, as is anticipated from the variation

in the extent to which the affected regions were dependent on the occluded artery for their blood supply.

When these analytical data are interpreted histochemically, the changes appear to involve an average increase of 15 per cent in the proportion of extracellular tissue, the equivalent of a 3 per cent fall in the relative mass of fibers, without significant change in the water or potassium concentration in the muscle fibers.

These conclusions rest on the assumption that chloride and sodium did not enter the muscle fibers as a result of the temporary ischemia. Support for this assumption may be found in the fact that *a*, approximately the same change in extracellular fluid was found whether sodium or chloride was used as a measure of the extracellular fluid, and *b*, the concentration of water in the fibers appeared to remain constant when calculated on this same assumption. Therefore, either chloride and sodium remained outside the fibers, as assumed, or, which seems less likely, chloride, sodium, and water must have entered the fibers in just the proportions existing in the extracellular fluid.

With these reservations, one may conclude tentatively that 4 or 5 hours after a short period of ischemia, there appears to be a moderate extracellular edema without evident change in the cardiac fibers. It is of interest that no abnormal morphological changes were detected either grossly or microscopically in these hearts (3).

Possibly this increase in extracellular fluid may be ascribed to damage to the small blood vessels of the heart. Tennant *et al.* (7) concluded that the capillary walls were altered so as to permit exudation following a 2 hour temporary arterial occlusion. Bronson (8) observed a marked increase in lactic acid in the myocardium within 1 or 2 minutes after the interruption of coronary blood flow. Other workers (19) (20) have concluded that acid production in conjunction with the release of specific vasodilators, was responsible for hyperemia subsequent to a temporary arterial occlusion. In this connection, it may be pointed out that either 15 or 45 minute occlusions resulted in an edema that was present 4 hours after the insult, whereas only occlusions lasting longer than 20 minutes produced permanent histological lesions (3). Conceivably, the shorter occlusions injure only the blood vessels, whereas the longer occlusions may injure both vessels and muscle fibers.

Analyses of hearts removed 24 hours or more after the termination of temporary arterial occlusions. In 4 instances, following the temporary occlusions, 1 to 16 days were allowed to elapse before the removal of the heart. In 2 of these instances, the occlusions lasted 20 minutes and the animals were allowed to survive 24 and 26 hours. No material changes were observed in the chemical constituents of either of these hearts.[3] These negative results are consistent with the general observations of Blumgart *et al.* (3) that there are seldom any permanent histological changes found in the myocardium following arterial occlusions of 20 minutes or less. It would appear that although an increase in extracellular fluid occurs following occlusions lasting only 15 or 20 minutes, this increase is only transitory and subsides within 24 hours after the operation.

[3] A minute area of necrosis, not obvious on gross examination, was found by Dr. M. J. Schlesinger on microscopic examination in one section of one of these hearts.

Temporary occlusions lasting longer than 20 minutes usually result in permanent morphological changes in the myocardium, detectable both grossly and microscopically. Two such cases were examined chemically (table 3). The arterial occlusions lasted 45 minutes and the hearts were removed 14 and 16 days postoperatively. At this time, the affected region (left ventricle) in both hearts showed a marked increase in water, chloride, and sodium. The potassium concentration was definitely decreased in the affected portion of the one heart for which values were obtained. Calcium was increased in both cases, particularly in one case in which a 70-fold rise occurred. These changes in water and electrolytes are similar to those observed by Wilkins and Cullen (21) in the hearts of human patients dying of cardiac failure.

The derived histochemical data for these two hearts indicate a relative increase in the extracellular tissue of the affected portions compared to the control

TABLE 3

Analyses of hearts removed 2 weeks or more after the termination of temporary arterial occlusions

$(H_2O)_c$, $(K)_c$ calculated per kilogram of fibers.
Other data based on a kilogram of fat-free, blood-free tissue.

			ORIGINAL DATA					DERIVED DATA				
			H_2O	Cl	Na	K	Ca	ECl	ENa	C	$(H_2O)_c$	$(K)_c$
			grams	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*grams*	*grams*	*grams*	*grams*	*mEq.*
No 4; 45 minutes occlusion; 14 days after occlusion	L.V.	Control	797	32.4	36.4	82.4	2.0	296	268	718	750	113
	L.V.	Affected	807	37.0	42.2	75.5	3.0	337	309	677	753	111
		Change	+10	+4.6	+5.8	−6.9	+1.0	+41	+41	−41	+3	−2
No. 5; 45 minutes occlusion; 16 days after occlusion	L.V.	Control	796.0	39.9	44.7		2.1	360	335	652	728	
	L.V.	Affected	805.5	46.5	56.1		154	422	420	579	728	
		Change	+9.5	+6.6	+11.4		+152	+62	+85	−73	0	

regions. Even the control regions of both hearts contained more extracellular tissue than normal. This change in the control portions might be correlated with the extra burden placed on the rest of the myocardium by injury to the region directly affected by the ischemia.

There was no difference in water concentration in the fibers of the control and of the affected regions, although in the second heart the water concentration in both control and affected regions was rather low. The possibility of increases in the amount of collagen fibers, especially in the affected regions, would have to be considered in deciding the exact water concentration in the fibers, since an increase in collagen, if not evaluated, would make the fiber water concentration appear too low.

The potassium concentration in the fibers was within normal limits. The calcium concentration was increased in the affected regions in both hearts. In the second heart (dog 5), the affected area contained calcium equivalent to 7.5 grams of $Ca_3(PO_4)_2$ per kgm. of cardiac muscle. This extra amount of

solids was subtracted from the other solids before calculating the water content of the fibers.

In both hearts calcium deposits were observed in sections used for morphological study.

The histochemical changes in the heart 2 weeks after a 45 minute coronary occlusion, therefore, appear to consist in part of a relative increase in the amount of extracellular tissue (or rather, a relative decrease in intracellular tissue) without definite changes in the composition of the remaining fibers. It is of interest that definite morphological changes were found in these hearts. The histological findings were those of healing myocardial necrosis (3).

Fibrillation and partial thrombosis. Two additional hearts were studied chemically. In one heart, the ventricles went into fibrillation $4\frac{1}{2}$ minutes after the beginning of the coronary occlusion and then into complete standstill within a few minutes. The electrolyte composition of this heart was within normal limits, and no histological changes could be detected.

In another case, after an operation planned as a control experiment, a thrombus was found which partially occluded the left circumflex coronary artery. This heart, which was removed 24 hours after the operation, showed chemical changes indicating the presence of an increased amount of extracellular fluid.

SUMMARY

1. Dog hearts were examined chemically at various times following the termination of temporary occlusions of a major coronary artery. Characteristic increases in the water, chloride, and sodium content of the myocardium were observed. In general, there was little change in the potassium concentration.

2. These changes have been interpreted histochemically as denoting an increase in the proportion of extracellular fluid without demonstrable change in the muscle fibers themselves.

3. Occlusions lasting 20 minutes or less produced an increase in extracellular fluid which persisted 4 hours but not 24 hours. Occlusions lasting 45 minutes produced a relative increase in the amount of extracellular tissue present both 4 hours and 2 weeks after the occlusion.

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SOME SPECIAL INSTANCES OF PREDOMINANT CHEMICAL STIMULATION OF THE EXPIRATORY HALF-CENTER

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The first change in breathing during pneumothorax is a powerful and more or less protracted inspiratory contraction which in due time is interrupted by a relatively weak expiratory contraction. From this point on both inspiratory and expiratory contractions grow in strength. With the first inspiratory contraction almost at maximum the expiratory contractions gain the more rapidly. As the intensity of inspiratory and expiratory components approximate each other, inspirations are interrupted at an earlier moment and the frequency of breathing is thereby accelerated. But later when the expiratory component gains dominance over the inspiratory act the expiratory act becomes disproportionately prolonged thus holding the inspiratory act in abeyance and retarding the frequency of breathing. The disproportionate increase of expiratory activity is apparently the critical factor involved in the changing events of breathing during pneumothorax. It is, therefore, significant that oxygen lack should play such an important rôle in the augmentation of expiratory activity (Gesell and Moyer, 1942). (See also figs. 2A and B of the present experiments.) The nature of the chemical stimulation associated with this phenomenon seemed worthy of further consideration.

RESULTS. *The nature and site of chemical stimulation of the expiratory half-center.* In figure 2A the dog was breathing room air when pneumothorax was established. Due to the relatively low sensitivity of the respiratory reflexes the progressive changes in breathing were small. Yet with the aid of a straight edge placed along the upper limits of the expiratory tracings during pneumothorax the increased expiratory activity becomes apparent. A few minutes later, with the dog now breathing an oxygen-poor mixture, the course of breathing during pneumothorax is greatly modified. The expiratory component grows more rapidly and breathing is more strikingly retarded. These effects of low oxygen occur with the greatest regularity and consequently are of interest in a comparison with the effects of other well-known forms of respiratory stimulation during pneumothorax.

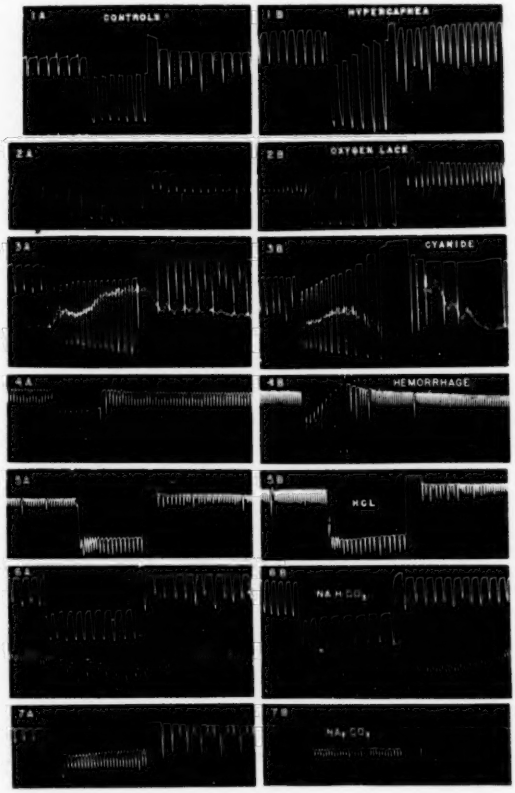
The first and foremost of these forms of stimulation is hypercapnia which exerts the same profound effect upon the respiratory response to pneumothorax as oxygen lack. Expiratory activity and retardation of breathing are markedly increased (see figs. 1A and 1B).

During cyanide poisoning, if the injection is sufficiently large, the intensification of expiratory activity and retardation of breathing may be exceptionally striking (see figs. 3A and 3B).

Hemorrhage also exerts a pronounced influence on the response of breathing to pneumothorax (see figs. 4A and 4B).

In figures 5A and 5B the effects of intravenous injection of hydrochloric acid are obvious. As the expiratory circumference diminishes frequency of breathing is retarded.

Last of the respiratory stimulants which we studied is sodium bicarbonate (see figs. 6A and 6B). It is to be noted first of all that bicarbonate actually



Figs. 1 to 7. Examples of expiratory augmenting action of asphyxia superimposed upon simple open pneumothorax. Breathing is recorded by the torso band method (inspiration is indicated by a down stroke). The left column of figures shows the changes of breathing produced by simple pneumothorax. They serve as controls for the right hand figures which show the effects of added asphyxias.

increases the breathing. (See the initial 5 breaths of fig. 6B and compare with those before pneumothorax in fig. 6A. Also compare the retarded pulse in fig. 6B with that of 6A.) Secondly, it is to be noted that the response to pneumothorax has been altered by sodium bicarbonate in a manner similar to that of hypercapnia, oxygen lack, cyanide, hemorrhage and hydrochloric acid. Intensified expiratory constriction is accompanied by greater retardation of breathing.

Only following administration of sodium carbonate does the frequency of breathing fail to be retarded during pneumothorax. Almost complete elimination of respiratory movements before the onset of pneumothorax is indicative of the high degree of hypocapnia produced by the injection. The well sustained accelerated frequency of breathing during pneumothorax is therefore thought to be significant.

Granting that the degree of retardation of breathing under our experimental conditions is an expression of the intensity of chemical stimulation of the expiratory half-center these results give promise of further information on the details of the chemical control of breathing, essential to the explanation of our present findings. Be it further conceded that cH plays a major rôle in the adjustment of breathing, the variety of forms of stimulation which we have studied offers singular opportunities to re-analyse the possible site of action of the hydrogen ion for, so far as we know, selective chemical stimulation of the expiratory half-center has never been investigated. Such a reconsideration from a new angle is all the more timely in the light of recent criticisms of the cH theory of respiratory control by Nielsen (1936), Krogh (1941) and Schmidt (1938, 1941)—containing many contradictions of a vital nature.

Surely there can be no doubt of a greater cH of the tissues and of the blood when combining pneumothorax with hypercapnia or intravenous injection of hydrochloric acid as compared with pneumothorax under otherwise normal conditions. There is more free acid present and the means of immediate elimination have been abolished. The increased stimulus of the expiratory half-center could, therefore, have been the increased cH of the blood or the increased cH of the nerve cells of the respiratory arc. Neither can there be any doubt of the state of the acid-base equilibrium existing during pneumothorax plus cyanide injection. Greater acid formation than normal added to the handicap of zero pulmonary ventilation must turn both the tissues and blood more acid than normal. The situation with hemorrhage is the same. A gradual accumulation of acid resulting from a subnormal volume flow of blood before pneumothorax plus a smaller sum-total of oxygen-saturated blood at the time of pneumothorax must assure a higher degree of tissue asphyxia. Both blood and tissues must turn more acid.

In the case of pneumothorax plus oxygen lack the universal increase of cH in tissue cells and blood is not so certain for cH determinations were not made. The possibility must be admitted that the blood may not have turned more acid than during pneumothorax following the breathing of room air due to the counteracting effects of a preceding reduction of oxyhemoglobin. On the other hand, it seems highly probable that the cells themselves did turn more acid than normal due to the rapid formation of anaerobic acid within them. This particular situation, therefore, might conceivably favor tissue cH as the possible site of action of respiratory adjustment as against blood cH.

The acid-base disturbances produced by sodium bicarbonate are much clearer in this respect. On theoretical grounds supported by experimental observations it may be safely inferred that intravenous injection of sodium bicarbonate is

capable of turning the interior of the cells more acid while at the same time lowering the hydrogen ion concentration of the blood. Direct experiment shows that cerebrospinal fluid, which is separated by a barrier membrane that blocks the free entrance of metallic ions from the blood, becomes more acid though the blood turns sharply alkaline (Gesell and Hetzman, 1926). Similar acid changes undoubtedly occur within the cells of the expiratory half-center when bicarbonate is injected. The greater inhibition of the heart during pneumothorax plus sodium bicarbonate (fig. 6B) is rather strong evidence that pertinent stations in the cardio-inhibitory reflex arcs turned more acid than during simple pneumothorax. Therefore, if it be either the cH of the blood or the cH of the nerve cells which determines expiratory activity the decision must be in favor of the latter.

The absence of expiratory excitation during pneumothorax plus the injection of sodium carbonate could accordingly be attributed to the alkaline state of the expiratory half-center caused by excessive diffusion of carbon dioxide out of the center into the blood.

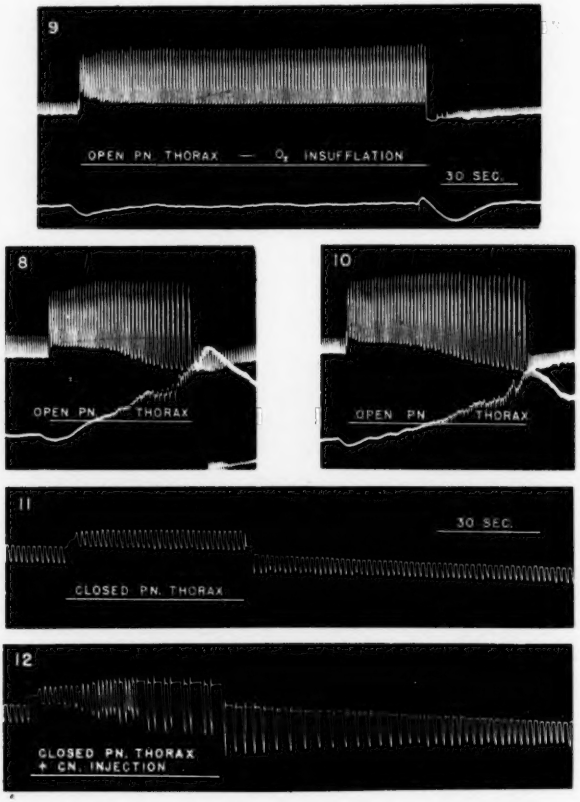
The cH gradient from cell to environment has also been considered as a factor determining nerve cell activity (see Andrus and Carter, 1924, and Andrus, 1924). A comparison of sodium bicarbonate and carbonate effects is pertinent on that score. Since both salts turn the blood more alkaline with respect to the cell and thus increase the outward gradient of cH they might be expected to have a similar action, yet their effects on breathing are diametrically opposite.

Another pertinent comparison is found in the effects of sodium bicarbonate and those of oxygen administration after a period of oxygen lack because of the probability that oxygen also produces opposite changes in the cH of the blood and tissues. However, in the case of oxygen it is the *blood* that turns acid and the tissues alkaline. Now if it is the cH of the blood that determines the degree of stimulation, breathing should increase. If, on the other hand, it is the cH of the tissues that matters, breathing should diminish as it does.

It is, therefore, clear that blood cH is at least not the determining factor in respiratory control, that an increase of free hydrogen in the blood (O_2 administration) can be incapable of increasing ventilation and that a decrease of free hydrogen ions in the blood (sodium bicarbonate administration) can be incapable of diminishing breathing. As we see it the explanation of chemical control of breathing rests primarily on cellular cH and *all* related factors affecting it (rate of acid formation, amount of acid in the cells and blood, the forces leading to movement of acid in either direction, the amount of buffers present, semipermeability of membranes, etc.). We believe that the failure of Nielsen, Krogh and Schmidt to recognize these facts in their entirety and the failure to appreciate that cellular and blood cH are not synonymous have led them into their present position.

Our first conclusion with regard to the origin of the increased expiratory activity during pneumothorax is that increased nerve cell acidity plays an important rôle. This conclusion receives additional support in figure 9 (please note that upstroke indicates inspiration in figs. 8, 9 and 10) in which asphyxia,

instead of being intensified during pneumothorax, is prevented by a gentle oxygen insufflation. Breathing then continues for an indefinite duration of time without the development of excessive expiratory activity such as seen in the control figures 8 and 10 where asphyxia is allowed. The steeply rising



Figs. 8, 9 and 10. The absence of augmentation of expiratory activity during open pneumothorax when asphyxia is prevented by gentle insufflation of the lungs with oxygen (fig. 9). In the control observations figures 8 and 10 where asphyxia is allowed excessive expiratory activity develops (inspiration is indicated by upstroke).

Figs. 11 and 12. The absence of development of excessive expiratory activity during closed pneumothorax when the animal's own breathing prevents the appearance of asphyxia (fig. 11). Addition of tissue asphyxia by the injection of cyanide initiates excessive expiratory activity and retardation of breathing (fig. 12). (Inspiration in fig. 11 and 12 is indicated by downstroke.)

blood pressure and the increasing cardio-inhibition show the intensity of asphyxia when insufflation is omitted.

The reflex factor of dominant expiratory activity. It is hardly probable that the slight pulmonary inflation produced by oxygen insufflation plays a signifi-

cant rôle in preventing excitation of the expiratory half-center. This follows from the respiratory response to closed pneumothorax when the chest is sealed after the lungs have collapsed during the first stages of open pneumothorax (figs. 11 and 12). Under these conditions both partial inflation and ventilation of the lungs obtain and breathing often reaches a steady state similar to that in figure 9 (see fig. 11). But if asphyxia is added to this situation by injection of sodium cyanide, the picture changes (fig. 12). Expiratory activity builds up to supernormal levels and retarded breathing comes on. Under ordinary conditions simple cyanide asphyxia produces a very rapid rate of breathing. What then is the modifying factor which creates this characteristic retardation of breathing?

Figures 13, 14 and 15 show that complete abolition of vagal reflexes by cold block does not prevent a progressive development of expiratory activity similar in general aspects to that already noted. If there is a proprioceptive factor involved it must be of extra vagal origin. Whatever this factor may be it fails to reveal its effects during vagal block if asphyxia is prevented by oxygen insufflation (fig. 15). A slight increase of expiratory activity will be noted which is commensurate, however, with the small degree of asphyxia (note the slowly rising blood pressure). It is interesting to note the effects of oxygen insufflation during vagal block in the absence of pneumothorax. There was an invariable reduction of expiratory activity, augmentation of rhythm and reduction of tidal air (figs. 14 and 15).

Head showed many years ago that blocking of the vagus nerves may produce a long series of slow, powerful and prolonged inspirations in which the torso is markedly stretched and the total ventilation is impaired. The causes of such breathing have been considered by Gesell and Moyer (1942) an example of which is illustrated in figure 16. The slowly rising blood pressure may be regarded as a reasonably safe index of a slowly mounting asphyxia and as this asphyxia increases expiratory activity grows stronger and longer until rhythmic breathing finally stops in a protracted and strong expiratory contraction. Deblocking of the vagi leads to recovery. Now this situation is different from all those mentioned above in that neither pneumothorax nor collapse of the lungs was essential for the development of excessive expiratory activity and yet there are two important points of agreement with pneumothorax which give it value in the present consideration. These are progressive asphyxia plus a well sustained over-distention of the torso capable of setting up new proprioceptive drives.

By the process of exclusion certain factors (pneumothorax, lung volume, lung immobility and vagal reflex activity) seem to be eliminated as important in the development of excessive expiratory activity and its associated retardation of breathing. Asphyxia and increased torso volume remain as the only invariable accompaniments of expiratory activity. Though simple open pneumothorax invariably gives rise to excessive expiratory activity its effects seem to resolve themselves into the factors of asphyxia plus increased torso volume. It, therefore, seems reasonable that any circumstances giving rise to a combina-

tion of these two conditions may be responsible for dominant expiratory activity. Precisely how these two conditions might function however is not known. Whether both are absolutely essential, whether one is more important than the other cannot be definitely concluded. It is conceivable that acidity

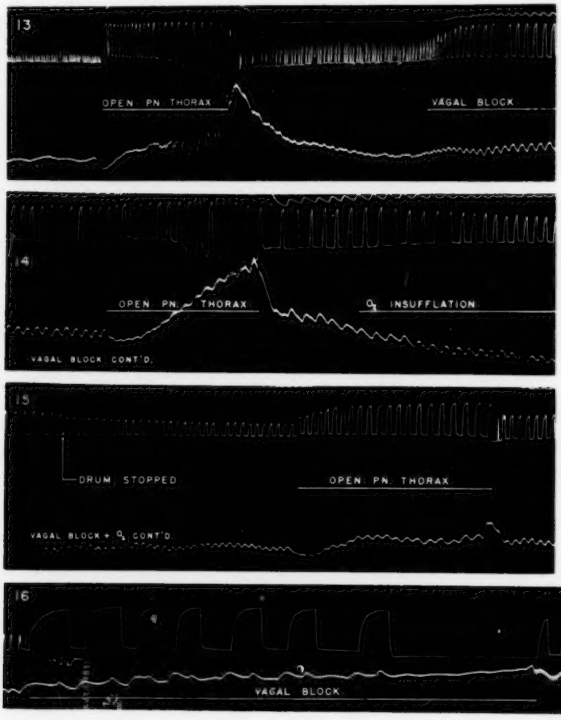


Fig. 13. Typical development of excessive expiratory activity during open pneumothorax with vagi functioning. Subsequent vagal block shows a deepening and slowing of breathing.

Fig. 14. Development of excessive expiratory activity during open pneumothorax and double vagal block. Oxygen insufflation subsequent to open pneumothorax decreases expiratory activity, increases frequency of breathing and diminishes tidal air.

Fig. 15. Oxygen insufflation during double vagal block diminishes the expiratory response to open pneumothorax.

Fig. 16. Simple vagal block which produces powerful, infrequent inspiration, plus asphyxia is accompanied by a progressive increasing dominance of expiratory activity. (Upstroke indicates inspiration in figs. 13, 14, 15 and 16.)

works directly upon the centers. All things considered, it appears more probable that the effects of these two factors are in some way interrelated. It is suggested that an increased torso volume initiates an expiratory reflex drive which in turn is intensified by asphyxia.

SUMMARY AND CONCLUSIONS

Special instances of predominant chemical stimulation of the expiratory half-center occurring during pneumothorax and simple vagal block were analysed in relation to the nature and site of chemical stimulation and in respect to a possible interaction of the chemical and proprioceptive stimulation.

Simple open pneumothorax increased the activity of the expiratory half-center and gave rise to a progressive dominance of that half-center over the inspiratory half-center. During such dominance the inspiratory half-center is held in increasing abeyance. Frequency of breathing diminishes in proportion to the increasing duration and strength of the expiratory act.

Asphyxia is definitely a most important contributing factor in the expiratory domination of breathing during pneumothorax for the addition of well-recognized forms of respiratory stimulation accelerate and increase the growth of expiratory activity. This was found to be true for hypercapnia, oxygen lack, cyanide, hemorrhage, hydrochloric acid and sodium bicarbonate.

Previous double vagal block did not prevent the occurrence of excessive expiratory stimulation during pneumothorax. If proprioceptive reflexes are essential they must consequently be extra vagal in origin.

When simple vagal block (chest intact) produced slow, powerful and prolonged inspiratory contractions and, therefore, deficient pulmonary ventilation expiratory activity also attained dominance of the respiratory act as asphyxia mounted. It is, therefore, concluded that neither pneumothorax nor vagal reflexes are essential to such dominance.

The two outstanding factors seem to be an increasing asphyxia and an associated modification of extra vagal proprioceptive drive possibly arising in the over distended torso.

It is tentatively suggested that asphyxia in some way increases the effectiveness of the incoming respiratory nerve impulses which under the conditions of our experiments have a high expiratory excitatory value.

The dominant chemical stimulation of the expiratory half-center allowed singular opportunities for a reanalysis of the nature and site of chemical stimulation. The evidence gathered by this new method of approach points to the importance of the rôle of the hydrogen ion and to an intracellular site of action.

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THE EFFECT OF SULFANILAMIDE ON THE ABILITY OF RABBITS AND DOGS TO WITHSTAND HIGH ALTITUDES

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The use of sulfanilamide as a chemotherapeutic agent prompted study of the effects of this drug on resistance to altitude. Flying personnel undergoing therapy with this drug are grounded, so that while the rationale of this precaution was not questioned, the effects of the drug on the functions of the organism were thought to deserve study. In addition, the advent of the air-ambulance rendered it advisable to determine, if possible, whether special precautions should be taken in such flights, if the patients have been receiving sulfanilamide.

METHODS. The animals were examined in a small decompression chamber, which was two feet in diameter and four feet in length. It was fitted with three windows and with numerous outlets. The outlets consisted of semi-couplings with removable plugs. They were utilized to permit sampling of arterial or venous blood or to obtain samples of alveolar air at various stages of decompression. They could also be used to record, outside the chamber, blood pressure, respiration, electrocardiograms, etc., or to inject into the animals any desired drug. An electrically-operated (Nash Hytor) vacuum pump permitted decompression of the chamber up to a rate comparable to ascents at 15,000 ft. per minute. Recompression could be accomplished in a few seconds. Coarse and fine inlet and outlet valves allowed accurate control. The chamber was made for the Department of Medical Research in the University of Toronto, by the Dominion Bridge Company.

When dogs were used, they were lightly anesthetized with nembutal, but no general anesthetic was given to the rabbits. In these animals the vessels were cannulated under local anesthetic with novocaine. When blood samples were taken, blood coagulation was prevented by heparin given intravenously.

Arterial blood samples were taken in rabbits by cannulating the proximal and distal ends of a common carotid artery. In dogs the femoral artery was employed. Blood from the proximal end of the vessel passed outside the chamber through a rubber stopper in one of the semi-couplings in one branch of a glass loop with a side-tube; in the other branch of this loop it returned through the same stopper to the distal end of the vessel. (See fig. 1A.) A continuation of the side-tube extended to a test-tube, where it ended beneath paraffin oil. This test-tube was connected with the interior of the chamber through another semi-coupling, so that the pressure within the tube could be rendered identical with that in the chamber. The side-tube was normally kept clamped; release of this clamp allowed the animal to bleed into the test-tube as the result of its own blood pressure. When adequate blood was obtained, the side-tube and the tube connecting the test-tube with the chamber could both be clamped. A tube

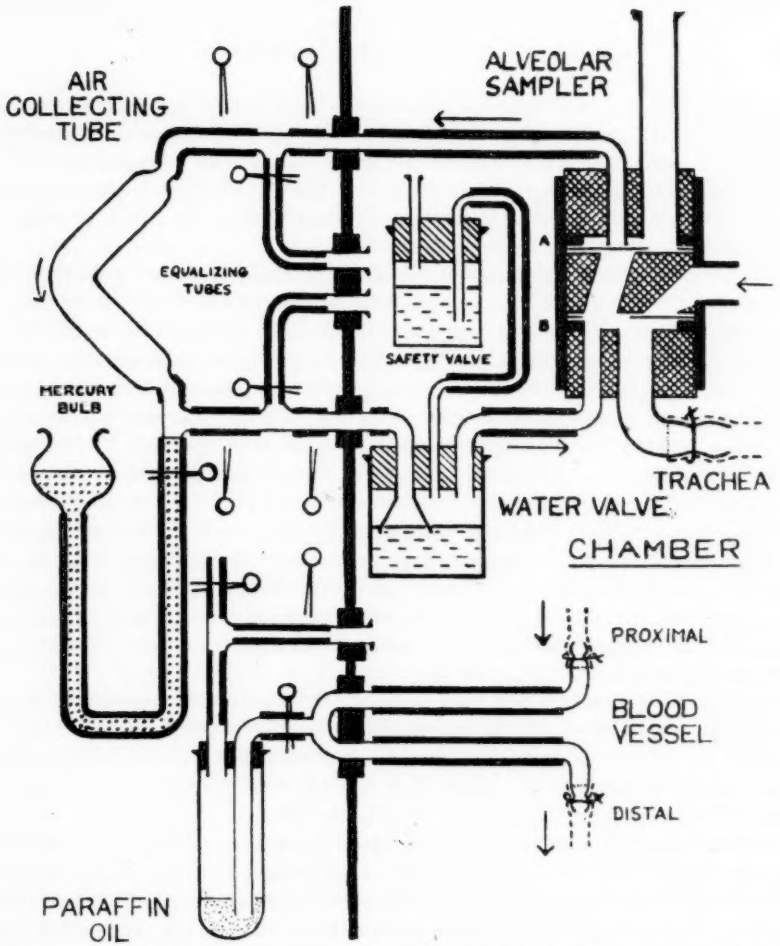

Fig. 1 A. Apparatus used for sampling alveolar air and blood from an animal while latter is decompressed to any simulated altitude. Parts to left of diagram are outside and parts to right are inside the decompression chamber, connections being made through semi-couplings in the chamber wall.

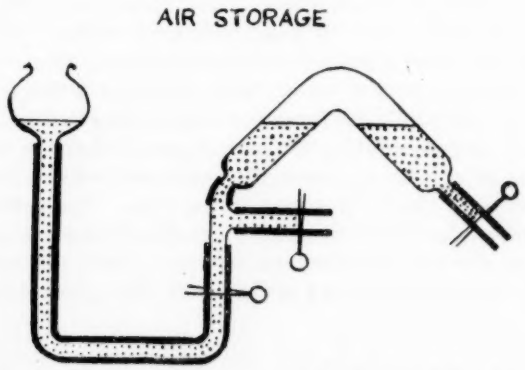

Fig. 1 B. Details of arrangement for storage of alveolar air.

connecting the test-tube with the outside air could then be opened and the tube be removed and replaced by a fresh tube. Before each sampling some blood was drawn and discarded to remove the blood stagnant in the side-tube (0.5 to 1.0 ml.). Precautions were taken to reduce heat loss from the exposed loops.

Venous blood samples were taken in a similar manner. The jugular vein was employed.

The blood samples so obtained could be handled by any of the accepted methods. The samples taken in the rabbits were 2 ml. in volume and the whole blood was analyzed for total CO_2 and O_2 content by the method of Van Slyke and Neill (1). In the dog experiments approximately 10 ml. were taken under paraffin oil into a tube with a constricted neck, and in most cases each sample was immediately centrifuged in a large cup filled with water at 38–39°C. Plasma was drawn off for CO_2 analysis while the blood was maintained at approximately this temperature. In some cases whole blood was used, and in this case was analyzed for CO_2 and O_2. Measurements were also made of the pH of either the plasma or of whole blood by the Beckman pH meter, utilizing a Beckman enclosed anaerobic glass electrode. The measurements were made when the sample was approximately at body temperature. Where corrections had to be made for the effect of temperature, an arbitrary correction of -0.005 pH per degree C fall in temperature was used. Hastings and Sendroy's phosphate buffer mixtures were used as standards.

Arterial blood CO_2 tensions were determined from the total plasma CO_2 and the plasma pH by means of the nomogram of Van Slyke and Sendroy (1, vol. II, fig. 41). In some experiments, where CO_2 content and pH determinations were made on whole blood, the plasma CO_2 content was calculated from the line chart of Van Slyke and Sendroy (1, vol. II, fig. 39).

Alveolar air samples were taken by an automatic sampler utilizing the principle described by Mackay (2). In brief, each inspiration draws into an air-collecting tube 1 to 2 ml. of air from the end of the previous expiration. Inspiratory and expiratory valves are placed as close as possible to a short, low tracheal cannula and the air is drawn from a side-circuit. This brief description of the principle may be clarified by reference to figure 1A. The automatic sampler was made from a short length of iron pipe with a hole drilled in one side, three rubber stoppers with their sides ground perpendicularly and each bored with two holes, two rubber washers (3 mm. thick) with valve flaps of very thin rubber sheeting affixed to them, and four lengths of glass tubing. The dead space between the tracheal cannula and expiratory valve was 4.5 ml. At each inspiration 2 ml. of the last expiration beyond this valve were drawn off into the air-collecting tube or tubes. These tubes completed the alveolar circuit outside the chamber and were connected to the parts inside through the semi-couplings. The air-collecting tubes of 25 ml. capacity were allowed to fill for several minutes, after which they were closed by strong spring clips. The contained air was brought to or above atmospheric pressure, by means of a mercury levelling bulb, before they were disconnected from the system. Since the collecting tubes were outside the chamber they could be changed at will. These collecting tubes

were curved, so that they could be sealed with mercury at both ends (see fig. 1B) until analysis of the contained alveolar air was convenient. All outside rubber to glass connections were made of pure heavy gum rubber, if they had to be made or broken at altitude. For other outside connections pressure tubing was adequate if it was cemented to the glass.

Alveolar air analyses were carried out with samples of approximately 1.5 ml. volume in the Van Slyke-Neill apparatus, employing a modification of the Van Slyke-Neill method for estimation of CO_2, O_2, and N_2 (1).

RESULTS. *The absolute altitude tolerance* of many rabbits was determined. The animals were tested by exposure to a lowering of pressure comparable to an ascent at 1000 ft. per minute. The limit of tolerance was marked usually by the occurrence of one prolonged or several repeated gasps, occasionally by simple cessation of breathing. At such times the pupils were widely dilated. If the pressure in the chamber was rapidly restored (commonly to ground level) within 20 to 30 seconds, usually the rabbits could be resuscitated and sometimes they recovered spontaneously. Complete apparent recovery of the animal was the rule within five minutes. It was possible, therefore, to test rabbits when under sulfanilamide treatment and to compare the results obtained in control runs on the same animals before or after recovery from the treatment. Such experiments were carried out on 8 rabbits with 18 observations when under treatment, and with 21 control runs. The rabbits received sulfanilamide by stomach tube before the altitude test, and were tested under conditions such that the concentrations of sulfanilamide in their bloods ranged from 5 to 90 mgm. per 100 ml. When under treatment the average altitude tolerance was 40,700 ft., while in control runs it was only 36,100 ft. Nineteen other control tests were made in 12 other rabbits and indicated an average tolerance of 36,600 ft. Armstrong and Heim (3) have reported a figure for rabbits of 37,235 ft. After sulfanilamide 72 per cent of the tested animals reached 40,000 ft. or over, while this was the case in only 10 per cent of all controls. Great haste in restoring the pressure at the critical point seemed less essential after the drug, as gasping tended to be more prolonged. Most of the sulfanilamide-treated animals recovered spontaneously and none died, while in the controls many had to be resuscitated and six died.

There was therefore undoubtedly an increased tolerance to altitude in the treated animals. Since abdominal distention seriously affects respiration at high altitude (4) it seemed possible that any increased tolerance might be dependent on a lowered appetite, if the animals had received sulfanilamide for one or more days. Another series of control tests (25) was therefore run on 17 other rabbits after they had been deprived of food (but not water) for 24 hours. The average height reached was similar to that of normal rabbits, namely, 37,400 ft. The animals were taken to their absolute limit, since in 24 per cent resuscitation was not achieved.

Tests were also made in rabbits receiving smaller doses of sulfanilamide in the therapeutic range. Twenty-four tests were made on 14 animals, to which a single dose of 50 mgm. per kilo had been given 30 minutes before the test.

Such a dose gave a blood concentration of 3 to 7 mgm. per 100 ml. The average height reached was 39,800 ft., when control runs averaged 37,500 ft. Distribution graphs in figure 2 indicate the data on these and the other groups.

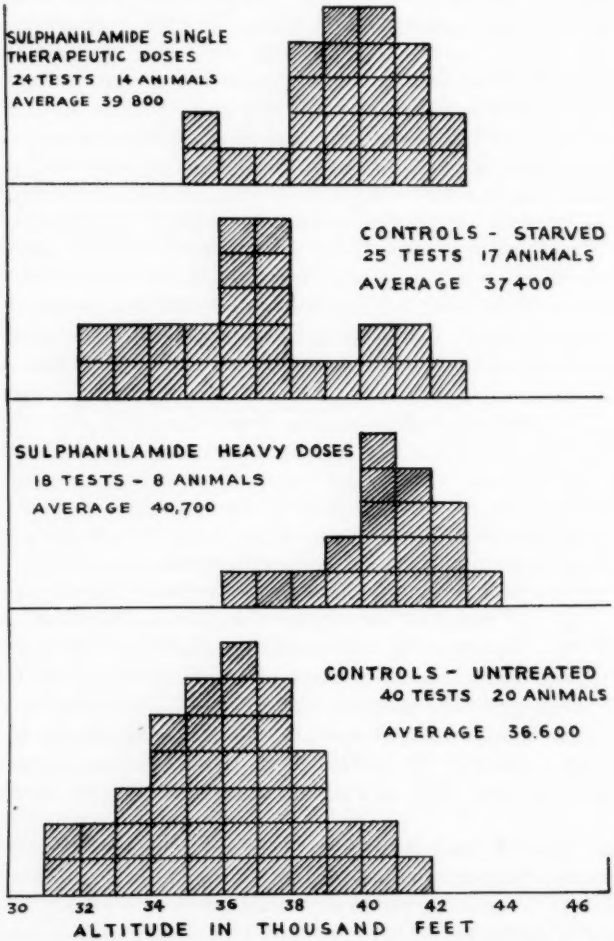

Fig. 2. Distribution graphs showing results obtained on decompressing sulfanilamide-treated and control rabbits to their absolute limit of tolerance at a rate corresponding to ascent at 1,000 ft. per minute.

The levels of oxygen and carbon dioxide in the arterial blood of rabbits during decompression were determined in many experiments. The animals were no doubt somewhat affected by the operative procedure in spite of the local anesthetic used. Thus in one control animal kept at ground level for a time similar

in duration to that of the experiments, the blood oxygen rose from an initial 19 to 20 volumes per cent and the carbon dioxide fell from an initial 41 to 34 volumes per cent. Such changes are unlikely to have interfered with the comparisons of the results obtained with and without sulfanilamide. In six control rabbits the initial average oxygen and carbon dioxide levels were respectively 15.7

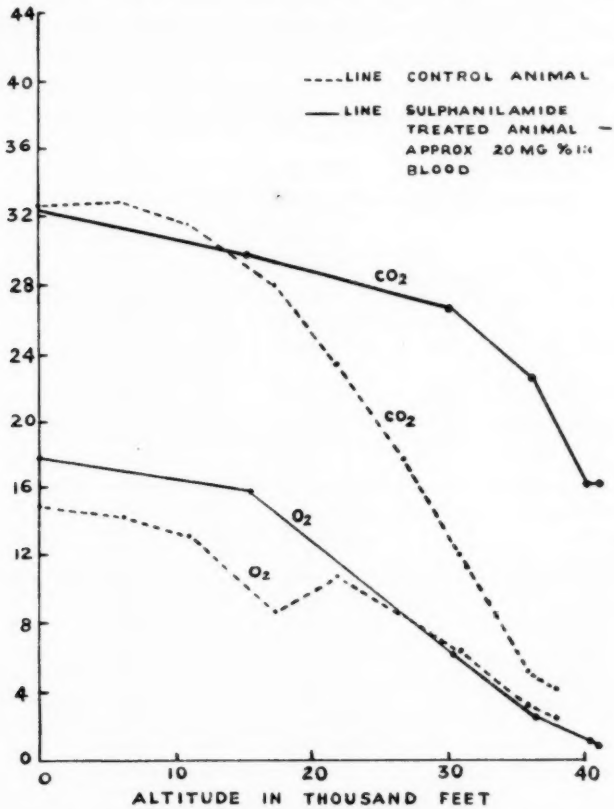

Fig. 3. Changes in blood CO_2 and O_2 content (in volumes per cent) of representative sulfanilamide-treated and control rabbits during decompression at a rate corresponding to ascent at 1,000 ft. per minute.

and 35.6 volumes per cent. Evidence of a fall in blood oxygen content appeared above 10,000 to 12,000 ft. and was accompanied by a fall in blood carbon dioxide, due to hyperventilation. At the limit of tolerance (38,400 ft.) the oxygen and carbon dioxide levels averaged 1.9 and 7.9 volumes per cent. In nine animals heavily dosed with sulfanilamide the average blood oxygen and carbon dioxide were 16.7 and 30.2 volumes per cent before decompression. The carbon dioxide values were therefore subnormal. During decompression the changes in oxygen

content resembled those of the controls, but the reduction in carbon dioxide was much slower. At the average limit of tolerance of 41,100 ft. the carbon dioxide of the blood averaged 13.7 volumes per cent. The fall in carbon dioxide was therefore much less than in the controls and the final values were higher, in spite of the initial low levels. The effects obtained in two animals are indicated in figure 3. The actual values obtained are given in table 1.

Three similar experiments were carried out on rabbits which received only 20 mgm. of sulfanilamide per kilo. All three animals started with a high arterial carbon dioxide level (averaging 44 vol. per cent) and attained very high altitudes (averaging 43,800 ft.), where the concentrations of carbon dioxide in the blood

TABLE 1

Blood O_2 and CO_2 during ascent at 1000 feet per minute

RABBIT	GROUND LEVEL, O_2	FINAL O_2	GROUND LEVEL, CO_2	FINAL CO_2	TOLERANCE
27—Control	16.9	2.3	26.7	5.5	35,000
26—Control	16.7	1.8	36.5	5.0	40,000
13—Control	15.5	1.6	37.8	6.2	38,500
14—Control	15.0	2.5	32.6	4.3	38,000
46—Control	12.9	2.3	38.7	5.8	37,000
47—Control	17.1	1.1	41.4	17.8	42,000
Average	15.7	1.9	35.6	7.4	38,400
4—Sulfanilamide	17.4	2.2	29.1	11.6	42,000
5—Sulfanilamide	13.7	0.9	32.2	17.8	43,000
21—Sulfanilamide	15.6	1.1	34.7	11.2	45,500
1C-9—Sulfanilamide	17.4	1.0	32.3	16.2	41,000
534A—Sulfanilamide	15.6	3.3	27.8	17.8	31,000
970A—Sulfanilamide	17.8	1.6	21.3	7.5	40,000
C40—Sulfanilamide	18.2	2.2	35.7	21.8	36,000
1C-22—Sulfanilamide	16.8	0.9	29.6	8.4	45,000
1C-20—Sulfanilamide	18.2	1.4	29.0	10.8	46,500
Average	16.7	1.6	30.2	13.7	41,100

were still high (averaging 12.8 vol. per cent). Such results suggest strongly an important influence of moderate carbon dioxide retention on the altitude attained.

The levels of oxygen and carbon dioxide in the venous blood of rabbits during decompression were only examined on a few occasions. In one rabbit both arterial and venous samples were taken. The arterio-venous differences for oxygen and carbon dioxide at ground level were 8.8 and 4.7 volumes per cent. At 41,000 ft. the arterio-venous oxygen difference had fallen to 1.5 volumes per cent, whereas that for carbon dioxide remained unchanged.

The levels of oxygen and carbon dioxide in the arterial blood during decompression and their relation to alveolar air were investigated in dogs. These animals were

examined at ground level, then exposed to a simulated altitude and then both sets of observations were repeated following a large intravenous dose of sulfanilamide. If the control arterial blood carbon dioxide tensions did not coincide closely with those found in alveolar air, the whole experiment was discarded. In those experiments where the results seemed entirely acceptable, no differences were found between these tensions at any altitude, either before or after sulfanilamide. The results of two experiments are summarized in table 2.

TABLE 2

Arterial blood-alveolar air CO_2 tension relationships

	TIME	SAMPLE	PRESSURE*	PLASMA pH	PLASMA CO_2	PLASMA CO_2 TENSION	ALVEOLAR CO_2 TENSION
			D-2				
			mm. Hg		*vols. per cent*	*mm. Hg*	*mm. Hg*
Before sulfanilamide	1128	I	751 (0)	7.35	46.4	37.5	36.0
	1201	II	281 (25,000)	7.56	34.5	16.9	17.1
	117	III	195 (33,000)	7.65	27.1	10.7	10.6
After sulfanilamide—	259	IV	751 (0)	7.52	33.0	17.6	15.5
blood level 20 mgm.	321	V	281 (25,000)	7.68	28.5	10.5	9.5
%	340	VI	195 (33,000)	7.69	28.4	10.4	9.3
	412	VII	751 (0)	7.53	27.8	14.5	14.6
	432	VIII	224 (30,000)	7.66	19.3	7.5	5.6
			D-13				
Before sulfanilamide	1112	I	759 (0)	7.41	37.2	25.3	24.4
	1130	II	345 (20,000)	7.46	30.7	18.4	17.2
	1144	III	225 (30,000)	7.47	21.2	12.5	10.4
After sulfanilamide—	118	IV	759 (0)	7.36	32.0	24.2	25.4
blood level 25 mgm.	140	V	349 (20,000)	7.46	28.6	17.4	14.3
%	151	VI	225 (30,000)	7.58	15.0	7.5	7.9
	201	VII	225 (30,000)	7.42	14.8	9.6	8.4
	319	VIII	759 (0)	7.47	25.7	15.3	16.8

* Numbers in parentheses indicate altitude in feet.

The possible relationship of methemoglobinemia to altitude tolerance when repeated doses of sulfanilamide had been employed received some investigation. Wendel (5) states that sulfanilamide does not produce methemoglobin in rabbits or dogs as it does in man (6). Consequently, methemoglobinemia was induced by intravenous injection of sodium nitrite. A number of experiments were made on rabbits in which sodium nitrite injections had reduced the oxygen capacity by 5 to 9 volumes per cent. A reduction of the altitude tolerance by some 3,000 ft. was found, if the standard rate of ascent of 1,000 ft. per minute was employed. This apparently slight effect became more evident if exposures to low oxygen

tensions of longer duration were used. Thus four rabbits received 60 mgm. of sodium nitrite per kilo, were taken to 25,000 ft. and were kept at this simulated altitude for 25 minutes. All four animals died, though such an experience has not harmed a single one of many normal rabbits.

Two dogs were used for additional tests. Repeated tests separated by at least two days were employed. The absolute limits of their altitude tolerances, during an ascent at 1,000 ft. per minute, were respectively 38,000 and 46,000 ft. Two hours after a dose of sodium nitrite of 30 mgm. per kilo had been given intravenously, the concentrations of methemoglobin in their bloods (determined by the loss in oxygen capacity) were equivalent to 13 and 8 volumes of oxygen per 100 ml. of blood. Their altitude tolerances were then 35,000 and 39,000 ft. During these latter tests signs of impairment of function, such as weakness of the limbs, loss of response to bell-ringing, and profound unconsciousness developed somewhat earlier than during the control tests. Even more marked was an earlier development of respiratory responses to anoxia. When methylene blue was injected intravenously (0.2 ml. of a 1 per cent solution was given per kilo of body weight) two hours after such a dose of sodium nitrite, the concentrations of methemoglobin were only equivalent to a loss of 1 and 3 volumes of oxygen per 100 ml. The altitude tolerances were not lowered; they were 42,000 and 45,000 ft.

In all these tests no evidence was found of any permanent damage. The dogs all recovered their normal posture and previous appearance within 5 to 15 minutes.

Methylene blue injections had been also employed after dosage with sodium nitrite in five rabbits. It appeared ineffective, but the later experiments in dogs suggest that the dosage used was probably inadequate.

Discussion. The results obtained may be briefly summarized to facilitate discussion. Sulfanilamide given to rabbits in heavy doses increases the resistance of rabbits to low oxygen tensions. If they be exposed to a simulated altitude at a rate of decompression equivalent to an ascent at 1,000 ft. per minute, the altitude they can tolerate is raised about 3,000 ft. This increased tolerance is associated with, and probably dependent on a relative retention of carbon dioxide in the blood. Though there is a fall in the content of carbon dioxide in the blood, it is not nearly as great as that observed in control animals. The question arises whether this retention of carbon dioxide is dependent on the known depression of carbonic anhydrase by the drug.

The effect of the drug, when given under the conditions of these experiments, in raising the altitude tolerance is so definite and consistent that it is clearly established. This is true even though the average limits of tolerance attained by the sulfanilamide-treated animals are by no means outside the limits of individual variation in rabbits. This is in agreement with observations on monkeys made by Fulton (7).

During the altitude tests many of the rabbits showed a head retraction which lasted a few minutes only and was usually seen at about 18,000 to 25,000 ft.

At 18,000 ft. there was also some loss in temperature control which allowed a sudden rise in the skin temperature of the ear (8). During the rapid descent, after the occurrence of gasping or other changes in respiration had caused the ascent to be reversed, a generalized involuntary muscular tremor might develop. This usually persisted for several minutes after ground pressure had been restored and frequently extended into gross convulsive movements. Sometimes the tremors commenced during the last few thousand feet of the ascent. For some unknown reason the tremors and convulsions appeared to be more frequent in animals treated with sulfanilamide.

In rabbits on exposure to decompression the changes in the gaseous content of the blood were often not simple. There was frequently a slight increase in the oxygen content at 20,000 ft., as compared with that found at a slightly lower altitude. This probably depended on the decreased tension of CO_2 in the lungs and blood. Just before the limit of tolerance was reached the respiration might be depressed and, in consequence, the content of CO_2 in the blood might rise above its lowest value. In spite of these minor complexities considerable differences were obvious when treated animals were compared with controls. Rabbits dosed with sulfanilamide showed, at ground levels, lowered concentrations of CO_2 in the blood, a change presumably due to hyperventilation. Such hyperventilation has been observed clinically in man (9). During the ascent the blood content of CO_2 fell much less in rabbits heavily dosed with sulfanilamide than it did in control animals. When this dosage was lighter the initial lowering of the CO_2 content of the blood was not seen, but the animals still withstood very high altitudes and also exhibited a fairly high retention of blood CO_2.

In the experiments on dogs the carbon dioxide tensions calculable from blood examination were compared with those found in samples of alveolar air. In the more successful experiments no discrepancies were found either before or after sulfanilamide. The control observations preceded those in the presence of the drug. There appeared to be a progressive fall in the content of CO_2 in the blood during the experiment, and no relative retention of CO_2 at high altitudes after sulfanilamide could be demonstrated. In such experiments, however, it is difficult to be certain that the blood samples taken are truly representative of blood in equilibrium with the alveolar air drawn, and to prevent the effects of progressive changes in the animal with anesthetic and experimentation.

The question of whether depression of carbonic anhydrase is the cause of the increased altitude tolerance is hard to answer. It could explain the effects observed in the rabbits, since it would allow an abnormal retention of CO_2 in the blood without any equivalent increase in the alveolar CO_2 concentration. The increased CO_2 within the blood would render it more acid and facilitate the unloading of oxygen from the blood. If such an increase in the CO_2 content of blood was associated with an equivalent increase in CO_2 in the alveolar air, this CO_2 would displace sadly needed oxygen (since the nitrogen concentration can only undergo minor alterations) and the uptake of oxygen by the blood would be

impeded. Any incapacity of the blood to liberate CO_2 from carbonic acid would prevent this rise of alveolar CO_2, so that the beneficial effects of CO_2 in the blood could be obtained, without the deleterious effect of raised CO_2 in the lungs. For this reason the observations of Binet, Strumza and Voghel (10) that the apnea which terminates acute anoxia occurs just as rapidly with a high blood CO_2 as with profound acapnia, need not be considered as in direct conflict with the data here reported. The conditions of their experiments were different. Even in the absence of sulfanilamide and its effects moderate retention of CO_2 may be beneficial in reactions to anoxia according to the observations of Gellhorn (11).

The fact that the CO_2 tensions calculable from blood examination in dogs are found to agree with those of alveolar air, even after sulfanilamide, gives no evidence that carbonic anhydrase activity is affected, but it does not exclude this explanation. As has been pointed out by Roughton (12), the drug would only slow the chemical processes dependent on this enzyme and not completely inhibit them Consequently the blood might progressively alter between withdrawal and examination. In the body the pH may have been lower and the carbonic acid concentration in the blood higher than would appear to have been the case as judged by later examination.

One line of evidence would appear to be against this attractive hypothesis. Sulfapyridine is said not to inhibit carbonic anhydrase (13). Eight of the rabbits which had been previously given small doses of sulfanilamide were also tested after a large dose (600 mgm. per kilo) of sulfapyridine. The average altitude reached was the same as that reached after the small therapeutic doses of sulfanilamide and 3,000 ft. higher than in controls.

The experiments so far discussed deal entirely with the acute effects of large or small doses of sulfanilamide on animals. Chronic dosage might produce quite different effects, but since the drug is said not to induce methemoglobinemia in either rabbits or dogs, intravenous injections of sodium nitrite were employed to produce these effects. There was no doubt that the altitude tolerances were lowered, though the animals were able to endure quite high "altitudes." This would appear to be due to the relative insensitiveness of the central nervous systems of these lower species to oxygen lack, as compared with man.

Methemoglobinemia is not presented as a reason why flying personnel should be grounded during sulfanilamide therapy. Sufficiently disabling nervous symptoms have been described by other authors and there is no evidence that these nervous symptoms are in any way related to methemoglobinemia. The rationale of the grounding of air personnel, who are under therapy with sulfanilamide, is not questioned, and it is unlikely to be related to the phenomena that have been described in this paper. On the other hand, conditions may be quite different if air ambulances are employed. The crew might be able to fly with ease at altitudes quite hazardous to their invalid passengers. This difference might be grossly exaggerated if these passengers had received sulfanilamide and had any degree of methemoglobinemia. It would seem advisable, therefore, to give methylene blue to sick or wounded men, who have been so treated, as recommended by Wendel (5), before they are transported by air.

SUMMARY

1. Sulfanilamide in moderate to heavy doses improves the altitude tolerance of rabbits by some 3,000 feet.

2. This improvement in altitude tolerance is associated with a retention of CO_2 in the blood, as compared with the untreated animal. The contents average about 33 per cent higher.

3. No significant differences in CO_2 tension of arterial blood and alveolar air were found at altitude after sulfanilamide administration, so that the cause of the above phenomena is yet unascertained.

4. Methemoglobinemia causes some reduction in altitude tolerance, but tolerance is restored to normal by methylene blue, which hastens the reconversion of methemoglobin to hemoglobin.

The writer wishes to thank Dr. A. C. Burton for kind assistance given throughout this investigation, to Miss J. Lang, who made for him some of the examinations of the blood gases, and Miss M. Wheatley, who made the determinations of the concentrations of sulfanilamide in the blood.

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THE MEASUREMENT OF EDEMA IN THE HEART-LUNG PREPARATION[1]

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The quantitative measurement of edema production in organs such as the heart and lungs is made difficult by the fact that in most experimental situations the weight of the organ cannot be measured before and after periods of changed conditions. When the heart-lung preparation is studied over some hours it is almost invariably found that edema of both heart and lungs occurs (1). It is important to be able to measure the amount of such edema for many reasons, but particularly in order to study changes in water and electrolyte changes in the heart muscle itself.

Several methods suggest themselves as possible means of estimating the edema of organs. *a*. If the organ weight-body weight ratio in the normal animal is known, and varies within sufficiently narrow limits, comparison of observed with predicted organ weight will provide a measure of the increase due to the experimental conditions. If it can be assumed that edema formation is the only process leading to significant weight changes, those changes are obviously a measure of edema production. *b*. If it be assumed that the cells of an organ are and remain chloride-free or nearly so, a calculation by the usual methods, of the extracellular water in excess of the normal, provides a measure of edema (extracellular). *c*. The actual weight changes in an isolated organ, over a period of perfusion, can be measured and the progress of edema formation thus determined. Such a measure involves the assumption that the weight change represents majorly an increase in intra- and extracellular water.

The assumptions underlying these three methods are not inherently improbable, and the measurements themselves can be carried out with considerable accuracy. Another method, *d*, has frequently been used in the past. It consists in a determination of tissue water content. It depends on the assumption that the solids in edema fluid are either negligible or low and constant. It is known that the protein content of edema fluid is variable. Moreover measurement of total solids in tissues is subject to relatively more error, in proportion to expected changes, than are measurements of chloride or total weight. This is true partly because the volatile organic matter which is lost depends upon the time of, and the precise control of the temperature during drying. Altogether it might be predicted, and it is found to be true, that water content is not a reliable measure of edema.

[1] An excerpt from a thesis submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1940.

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METHODS. Hearts and lungs from apparently healthy mongrel dogs of both sexes were used in these studies. The animals were weighed with an accuracy of 0.1 kgm. or less. Control values for ventricular weight, lung weight and chemical analyses were obtained on 67 "normal" animals sacrificed by rapid bleeding from the carotid under ether anesthesia.

The development of edema was studied in 85 hearts and lungs after various periods following preparation of the Starling heart-lung system. The preparation was removed from the chest and immersed in a constant temperature bath as described by Peters and Visscher (2). At termination of the period of observation the atria were separated from the ventricles by cutting along the atrioventricular groove. Blood was removed by blotting with dry filter paper and the fresh ventricular weight determined by means of a torsion balance. The fresh lung weights were similarly determined after trimming off the visible bronchi flush with the lung parenchyma.

The tissue analyses were carried out on duplicate samples of ventricular muscle. Visible fat and connective tissue were trimmed off, and the whole ventricles run twice through a meat chopper, after which suitable samples were taken for analysis. Tissue and blood serum chloride analyses were carried out by method of Van Slyke and Sendroy (3). The water content of blood and tissue was determined by weight differences produced by drying for 48 hours in an oven kept at from 95° to 105°C.

RESULTS. The data from all observations on the ventricular weight plotted against body weight are shown in figure 1. The values for lung weight are plotted in figure 2. In each figure the solid points show data for normals and the open circles for heart-lung preparation organs. It is apparent that there is considerable overlapping of values in the case of ventricular weight but that there is virtually complete absence of overlap in the values for lung weight at a given body weight in the two groups. It should be pointed out that the absence of values for organ weights from heart-lung preparations at the higher body weights is due to the fact that no animals above 20 kgm. weight are used for heart-lung preparations because the apparatus employed will not accommodate such large hearts.

The average and extremes of ventricular weight/body weight and lung weight/body weight ratios of normal dogs of various weight ranges and of all the heart-lung preparations are given in table 1. The standard deviation from the mean ratios calculated from the entire normal series was ± 0.121 per cent of the body weight for the ventricular weight/body weight ratio and ± 0.080 per cent of the body weight for the lung weight/body weight ratio. The average normal ratios are in reasonable agreement with similar values reported by Stewart (4) and Herrmann (5). A significant variation of these ratios with the body weight is not evident in this series.

Both the average ventricular weight/body weight and lung weight/body weight ratios from the heart-lung preparations are significantly greater than the normal average ratios. Comparison of the average heart-lung and normal ratios indicates that the average increase in weight (edema) of the heart-lung

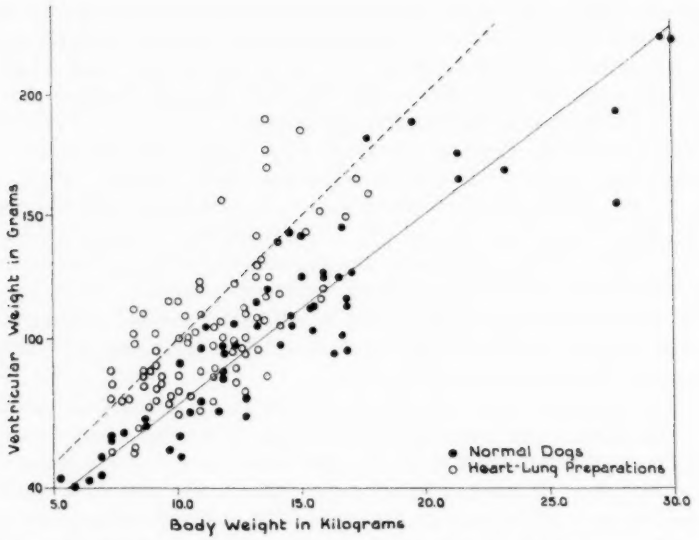

Fig. 1. The relationship of ventricular weight to body weight in normal dogs and in heart-lung preparations. The solid line represents the average ventricular weight/body weight ratio of normal dogs (0.758 ± 0.121). The broken line represents the average normal ratio (0.758) plus twice the standard deviation (± 0.121).

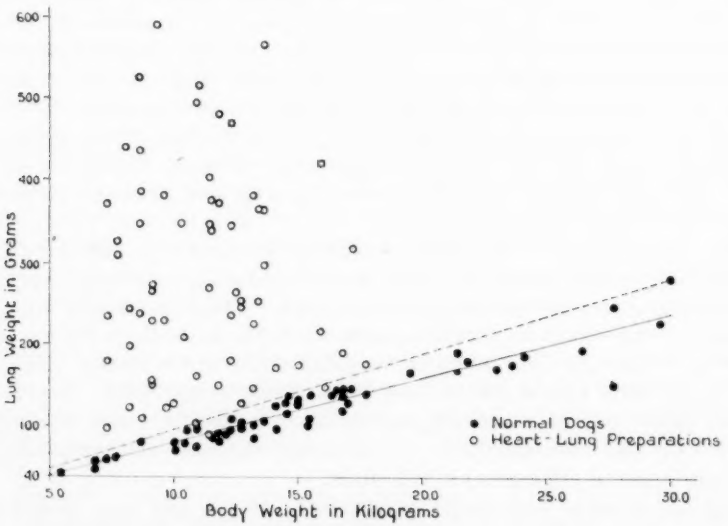

Fig. 2. The relationship of lung weight to body weight in normal dogs and in heart-lung preparations. The solid line represents the average lung weight/body weight ratio of normal dogs (0.796 ± 0.080). The broken line represents the average normal ratio (0.796) plus twice the standard deviation (± 0.080).

ventricles at the end of the preparation was 18 per cent and of the lungs 60 per cent of the final organ weight.

The increase in weight (edema accumulation) of 4 blood-perfused Langendorff dog heart preparations (6) has been recorded continuously for the duration of the perfusion period. An attempt to minimize the variations in the weight of the heart due to variations in the volume of the intravascular perfusion fluid has

TABLE 1

Ventricular weight–body weight and lung weight–body weight ratios

DOG WEIGHT RANGE, KGM.	(NO.) DOG WEIGHT KGM. (EXTREMES)	(NO.) VENTRICULAR WEIGHT GM. (EXTREMES)	(NO.) LUNG WEIGHT GM. (EXTREMES)	PER CENT OF BODY WEIGHT	
				(No.) Ventricles (Extremes)	(No.) Lungs (Extremes)
Normal dogs					
5–10	(17) 8.38 (5.46–10.90)	(17) 61.8 (40–96)	(11) 69.6 (44–93)	(17) 0.743 (0.530–0.953)	(11) 0.806 (0.720–0.936)
11–15	(25) 13.50 (11.10–15.90)	(25) 106.6 (69.0–142.5)	(21) 107.3 (82.0–138.0)	(25) 0.783 (0.543–0.987)	(21) 0.798 (0.652–0.938)
16–20	(12) 17.69 (16.35–20.90)	(10) 128.7 (94.0–189.0)	(11) 145.5 (119.0–175.0)	(10) 0.749 (0.575–1.030)	(11) 0.819 (0.707–0.878)
21–30	(12) 25.00 (21.36–30.00)	(7) 185.0 (145.0–223.0)	(11) 197.0 (150.0–282.0)	(7) 0.720 (0.523–0.823)	(11) 0.785 (0.545–0.970)
Complete series averages:	(67) 14.92 (5.46–30.00)	(59) 106.5 (40.00–223.0)	(54) 125.7 (44.0–282.0)	(59) 0.758 (0.523–1.030)	(54) 0.796 (0.545–0.970)
Heart lung preparations					
7–18	(85) 10.3 (7.3–17.7)	(85) 105 (54–190)	(65) 213 (91–590)	(85) 0.920 (0.607–1.390)	(65) 2.068 (0.780–6.125)

been made by measuring the weight of the preparation at zero perfusion pressures at the beginning and the end of the experiment. During the experiment the perfusion pressure was maintained at constant level. Weight changes due to variations in the volume of the vascular bed are undoubtedly small in comparison to the total changes in weight which were found (6).

Plotting the increase in weight of the heart against the duration of the preparation shows that over 90 per cent of the weight gain occurs during the first 30 minutes. After this initial rapid weight gain the weight of the heart increases

only very slowly for the duration of the preparation. Somewhat similar weight gain curves have been obtained by Ort and Markowitz (14) using the Langendorff rabbit heart preparation.

The heart and blood serum of the perfusion fluid were analyzed for chloride. The edema content of these hearts has been calculated[2] from their chloride content, on the assumptions that the muscle cells retain their "normal impermeability" to chloride and that the edema is extracellular. The average normal ventricular and serum chloride values which were used in the calculations were obtained from a series of normal dogs dispatched by hemorrhage while under ether anesthesia. These values are reported elsewhere (17).

Table 2 shows a comparison of the calculated edema values with the values determined by direct weight measurement. The agreement that was obtained is satisfactory and in all probability within the limits of error of this type of experiment.

TABLE 2

Edema formation in blood-perfused Langendorff dog heart preparations

HEART WEIGHT BY TORSION BALANCE		CHLORIDE CONTENT		FINAL EDEMA CONTENT	
Initial	Final	Heart	Serum	Weight method	Chloride method
grams	*grams*	*mgm./100 grams*	*mgm./100 grams*	*grams/100 grams of final heart weight*	*grams/100 grams of final heart weight*
231	374	277	506	39.3	39.1
106	170	287	506	38.7	41.7
134	204	312	550	34.3	41.6
121	157	245	575	22.9	22.7
Average 148	226			33.8	36.3

The edema content of 23 heart-lung ventricles has been calculated from ventricular chloride analyses, and of 33 ventricles from ventricular water contents. A check on the adequacy of these two methods can be gained by comparing the ventricular weight-body weight ratios of normal dogs with the ratios obtained in the heart-lung ventricles with and without correction for edema (table 3). The average ratio of the heart-lung ventricles after correction for edema by the

[2] The derived equation for the calculation of edema by the "chloride space method":

$$E = 100\ [Cl_v - Cl_n(Cl_s/Cl_{ns})] \div [Cl_s - Cl_n(Cl_s/Cl_{ns})]$$

E = Edema content in grams per 100 grams of final ventricular weight
Cl_v = Final ventricular chloride in milligrams per 100 grams of fresh tissue
Cl_n = Average normal ventricular chloride in milligrams per 100 grams of fresh tissue
Cl_s = Final serum chloride in milligrams per 100 grams of serum
Cl_{ns} = Average normal serum chloride in milligrams per 100 grams of serum.

A similar relation can be used to calculate edema from tissue water analyses:

$$E = 100\ (H_2O_v - H_2O_n) \div (H_2O_E - H_2O_n)$$

E = Edema content in grams per 100 grams of final ventricular weight
H_2O_v = H_2O content of ventricles in grams per gram of fresh tissue
H_2O_n = H_2O content of normal ventricles in grams per gram of fresh tissue
H_2O_E = H_2O content of the edema fluid in grams per gram of edema fluid.

chloride method is approximately normal. The average ratio is still considerably above normal after correction for edema by the water content method, indicating that this method does not adequately correct for edema.

Reference to figure 2 shows that a reasonably accurate value for the amount of lung edema occurring in a preparation can be determined by comparing the

TABLE 3

Average ventricular weight–body weight ratios

	NORMAL DOG VENTRICLES	HEART-LUNG VENTRICLES	HEART-LUNG VENTRICLES AFTER CORRECTION FOR EDEMA BY		
			H_2O method	Chloride method	Lung edema method
Number	(59)	(85)	(33)	(23)	(63)
Average	0.758	0.920	0.852	0.732	0.715
Extremes	(0.530–0.955)	(0.607–1.590)	(0.599–1.292)	(0.585–0.908)	(0.485–1.000)

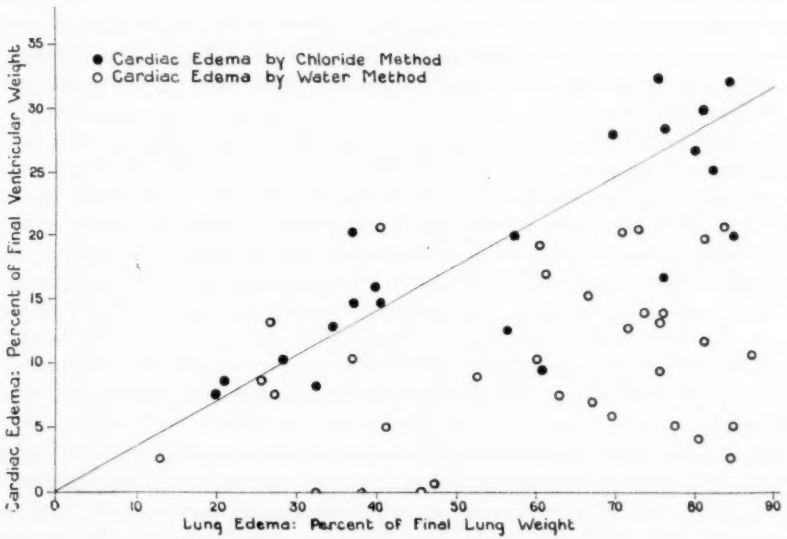

Fig. 3. The relationship between lung and cardiac edema in the heart-lung preparation The solid line is an arbitrary correlation line which was used to approximate the cardiac edema of heart-lung ventricles of unknown chloride content.

final lung weight with the initial lung weight predicted from the average normal lung weight/body weight ratio.

In heart-lung preparations, edema of both organs presumably results from similar causes, such as toxic substances in the blood and increased venous pressure. A correlation was therefore sought between cardiac edema (chloride method) and lung edema (weight measurement). The results are shown in figure 3. A positive correlation is evident between the relative amounts of

lung edema and of cardiac edema as calculated by the chloride method. A correlation between lung edema and the cardiac edema as calculated from water analyses is not evident; an additional indication that the magnitude of edema formation can not be measured adequately by means of water analyses.

Using the correlation found between lung edema and "cardiac chloride edema," the cardiac edema of 65 heart-lung ventricles has been estimated from the lung edema which occurred in these preparations. The adequacy of this method for the estimation of cardiac edema is also indicated in table 3. The average heart weight to body weight ratio obtained after edema correction by this method is somewhat below the normal ratio, indicating an overcorrection for the edema. This overcorrection was expected since several experiments, in which the cardiac-lung edema correlation was far removed from the other experiments, were omitted when the arbitrary correlation line was drawn through the experimental points. In these experiments which have been omitted it has been observed that the lung edema occurred early in the preparation and was of a very rapidly developing massive type which caused an early death of the preparation. This type of lung edema is associated with relatively very small amounts of simultaneously occurring cardiac edema and perhaps the underlying mechanism is of a different type than that which causes the pulmonary edema usually seen in this preparation.

Discussion. Obvious lung edema has been known to occur in most heart-lung preparations but quantitative studies of this phenomenon have previously been lacking. The cause of this lung edema is not clear. The earlier investigators (7, 8, 9, 10) included such physical factors as high pulmonary arterial and venous pressures, high rates of blood flow, and decreased osmotic pressure, cell volume, and viscosity of the blood as determining causes of pulmonary edema of this type. More recently Lambert and Gremels (11) and Newton (12) have shown that some toxic factor in shed blood which appears and increases as the blood stands is the chief causative factor of the pulmonary edema. Code, Evans and Gregory (13) demonstrated that this toxic factor is in all likelihood not histamine.

The present study shows that cardiac edema also occurs to a greater or less degree in all heart-lung preparations, presumably due to the action of the same factors which result in the accumulation of edema fluid in the lungs. Ort and Markowitz (14, 15) have shown that hearts perfused through the coronary arteries gain from 20 to 150 per cent of their original weight during the first hour of perfusion depending upon whether heparinized blood or Locke's fluid was used as the perfusing fluid. Peters and Visscher (2) have reported increases of 20 per cent in the volume of ventricular musculature of the heart-lung preparation during a period of 25 minutes under special conditions.

The average amount of cardiac edema which has been found to occur in the heart-lung preparation is of such magnitude as seriously to compromise any experimental results obtained from this preparation which are expressed in terms of the final fresh or dry heart weight. This statement probably applies to most isolated perfused preparations.

The inadequacy of the correction for edema on the basis of tissue water determinations arises from the fact that the edema fluid in this type of preparation has a relatively high dry weight content which cannot be readily determined. Water content determinations on the fluid which exudes from obviously edematous heart-lung hearts upon standing give average values of 90 per cent as compared to the average plasma value of 93 per cent. Such hearts not uncommonly have a normal water content although chloride analyses indicate that over 20 per cent of their weight is edema fluid.

The results indicate that the cardiac edema which occurs in the heart-lung preparation can be estimated with reasonable accuracy as an increase in the "extracellular water"[3] content. Therefore this edema appears to be for the most part extracellular. Permeability of dead or injured muscle cells to chloride should cause a positive error in the extracellular water calculation, but intracellular edema, which would not be included in edema calculated from chloride analyses, would tend to cancel this error.

Evidence from potassium, sodium, chloride and water analyses of heart-lung ventricles (18) indicates that their intracellular electrolyte and water pattern is essentially normal. The "extracellular water" content of heart-lung ventricles is, however, much larger than normal, indicating that the site of edema formation is extracellular. The factor or factors which cause the marked alteration in capillary permeability resulting in edema formation in the heart-lung preparation evidently do not affect the existence of the electrolyte concentration gradients which are normally present across the cardiac muscle cell membrane.

The external ventricular volume of the majority of the heart-lung preparation hearts was recorded throughout the experiment by means of a Henderson-type cardiometer. It is the opinion of the authors that this procedure considerably increases both cardiac and lung edema formation in this preparation.

SUMMARY

1. The average ventricular weight/body weight, and lung weight/body weight ratios from eighty-five heart-lung preparations have been compared with similar ratios from normal dogs. The differences found in the average ratios indicate that on the average 18 per cent and 60 per cent of the final heart-lung ventricular and lung weights respectively are edema fluid.

2. Satisfactory agreement was found between the edema calculated from cardiac chloride analyses and the edema determined by the weight increase of four blood-perfused Langendorff dog hearts.

3. The average ventricle weight/body weight ratios of 23 heart-lung hearts corrected for edema on the basis of chloride analyses was not significantly different from normal.

4. The edema content of heart-lung hearts cannot be adequately corrected for edema on the basis of tissue water content. Concentrations of tissue con-

[3] "Extracellular water" has been used (16) as a non-committal term for the calculated chloride space, since this calculated value is actually neither the "chloride space" or "extracellular space" but only a good approximation of these two phases of tissue.

stituents of this or similar preparations are of little significance if expressed on a dry or a wet weight basis.

5. In the majority of heart-lung preparations a positive correlation exists between the relative magnitudes of cardiac and lung edema which occurs. This constitutes an indication that similar factors are responsible for the edema formation in the two organs.

6. The major part of the edema fluid which accumulates in heart-lung hearts appears to be extracellular in position.

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ELECTROLYTE AND WATER CONTENT OF THE VENTRICULAR MUSCULATURE OF THE HEART-LUNG PREPARATION WITH SPECIAL REFERENCE TO THE EFFECTS OF CARDIAC GLYCOSIDES[1]

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The studies of Harrison and his co-workers (1, 2) have demonstrated that heart failure in humans is associated with changes in the electrolyte composition of the cardiac musculature. The potassium content of hearts from patients dying of heart failure was found to be decreased. A similar change was found in the hearts of dogs receiving toxic doses of digitalis (3). More recent studies have demonstrated that toxic doses of digitalis cause a decrease in the potassium content of striated muscle (4), the ventricular musculature of the heart-lung preparation (5), the Langendorff perfused rabbit heart (6), and isolated strips of the ventricular musculature of the turtle (7). The reported effects of "therapeutic doses" of digitalis bodies on the potassium content of heart muscle are controversial. Calhoun and Harrison (3) reported a slight decrease of questionable significance in the cardiac potassium content of the intact dog, the blood potassium findings of Wood and Moe on the heart-lung preparation (5, 8) indicated a loss of potassium from the heart and/or the lungs, Wedd (7) could demonstrate no change in potassium content of the heart of the intact cat or in isolated strips of the turtle heart, Hagen (6) found the potassium content of Langendorff perfused rabbit hearts was increased after "therapeutic doses" of Lanatoside C, and Boyer and Poindexter (9) found that therapeutic digitalis dosage increased the potassium content of the hearts of intact cats.

Results of potassium, sodium, chloride and water analyses of the ventricular musculature of failing heart-lung hearts with and without treatment with cardiac glycosides are reported in this paper.

METHODS. The heart-lung preparations were set up as described by Peters and Visscher (10) so that oxygen consumption and external diastolic volume could be recorded continuously, and the measurements necessary for the calculation of the external work and hence the external mechanical efficiency of the preparation could be made at suitable intervals.

The preparation and analysis of the ventricular musculature were carried out as described by Wood and Moe (11) and Wood (12).

RESULTS. The ventricles of forty heart-lung preparations receiving none or

[1] An excerpt from a thesis submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1940.

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various doses of digitalis glycosides[2] have been analyzed for potassium and water. Some of these hearts have also been analyzed for sodium and chloride.

The average values and the range of variation in the potassium and water analyses are given in table 1. The "digitalis hearts" are subdivided according to whether they received "toxic" or "therapeutic" doses of a digitalis glycoside. In this paper toxic and therapeutic doses are defined in reference to the heart-lung preparation in accordance with Moe and Visscher (13). A toxic dose of a digitalis glycoside is one which produces cardiac arrhythmias in the heart of the heart-lung preparation. A therapeutic dose is one which will increase the external mechanical efficiency of the heart-lung without the production of cardiac arrythmias within a period of 150 minutes after administration of the drug.

TABLE 1

Heart-lung ventricles, average values

(NO.) HEARTS OF	WATER CONTENT PER 1000 GRAMS FRESH TISSUE	POTASSIUM CONTENT PER		DURATION OF PREPARATION	EDEMA CONTENT OF VENTRICLES AS CALCULATED BY		K CONTENT PER 1000 GRAMS "EDEMA CORRECTED"†, FRESH TISSUE
		1000 grams fresh tissue	100 grams of dry weight		Lung edema method*	H_2O content method*	
	grams	*mM*	*mM*	*minutes*	*grams per cent*	*grams per cent*	*mM*
(15) Normal dogs	783 (766–799)	81.5 (73.4–87.0)	37.4 (31.6–40.0)				81.5
(12) Control Heart-lungs	799 (784–825)	71.6 (62.1–85.9)	35.6 (32.3–42.3)	183 (104–255)	20.0 (12.5–28.7)	10.6 (0.65–23.8)	88.5
(7) Therapeutic dose Heart-lungs	799 (781–812)	63.1 (49.2–75.0)	31.6 (27.2–33.8)	249 (147–316)	25.0 (15.7–32.2)	10.6 (0.0–18.8)	83.2
(15) Toxic dose Heart-lungs	795 (781–810)	59.2 (44.6–71.9)	28.2 (20.6–33.6)	185 (92–330)	21.5 (7.6–30.2)	7.8 (0.0–17.5)	72.4

* Wood and Moe (1941).

† Calculated by lung edema method (Wood and Moe, 1941).

Without chloride analyses there is no adequate means of correcting for edema in heart-lung hearts. Table 1 includes the amount of cardiac edema as calculated by the water content and lung edema methods, the only available means of estimating the edema content in this group of hearts since they were not analyzed for chloride (11). The actual cardiac edema probably correlates more closely with the calculated edema based upon the simultaneously occurring lung edema (11). Therefore the ventricular potassium content has been computed on the basis of edema corrected (lung edema method) fresh tissue weight in an effort to gain a better idea of the exchanges occurring in the actual cardiac tissue. The inadequacy of this correction is shown by the fact that these calculations (table 1) show higher potassium concentrations in control and thera-

[2] The pure glycosides of *Digitalis lanata*, Lanatosides A, B, or C, were used in the majority of these experiments.

peutic dose hearts than are obtained with normal hearts. Average edema values as obtained by this method have been shown to overcorrect for edema (11).

In spite of the difficulty of interpretation due to cardiac edema, several definite indications can be drawn from table 1: 1, heart-lung ventricles have an increased water content, presumably due to the accumulation of edema fluid; 2, with the doses used in these experiments the magnitude of this increase is apparently not affected by digitalis glycosides; 3, all heart-lung ventricles have a decreased potassium concentration compared to the normal dog heart when concentrations are expressed on either a fresh tissue or a dry weight basis; 4, hearts receiving toxic doses of digitalis glycosides show a much greater decrease in potassium concentration than the control heart-lung hearts; this decrease is much too large to be explained on the basis of edema content; 5, hearts receiving therapeutic doses of digitalis glycosides show a greater decrease in potassium concentration than do control hearts. However, it must be noted that the average duration of the preparation was about one-third greater in the therapeutic dose digitalis experiments than in either the controls or the toxic dose experiments. This greater time would favor spontaneous potassium loss and edema formation. The correction for edema leaves a small apparent loss of potassium in comparison with control hearts which is slightly less than one-third that found with toxic doses of digitalis glycosides.

More decisive information can be derived from the hearts which were analyzed for sodium and chloride in addition to the potassium and water analyses. The average values and range of variation of these analyses are given in table 2. The wet and dry weight potassium and water concentration changes in this group of hearts are similar to those shown in table 1. The chloride concentration in heart-lung ventricles is increased; the magnitude of this increase is apparently not significantly affected by the doses of digitalis glycosides used in these experiments. The sodium concentration in heart-lung ventricles is likewise increased; the magnitude of this increase is significantly larger in preparations which received a toxic dose of a digitalis glycoside.

The amount of cardiac edema occurring in this group of hearts was calculated from both the sodium and chloride analyses as described by Wood and Moe (11). A knowledge of the edema content of the heart makes possible the calculation of the approximate exchanges which occurred in the actual ventricular musculature. The average results of some of these calculations are given in table 3.

The average edema contents of the control heart-lung ventricles as calculated either from the sodium or chloride concentrations were not significantly different and amounted to 17.5 grams per cent of the final ventricular weight. The potassium content when calculated on the basis of edema corrected fresh tissue was not significantly different from normal, 80.0 mM per kilo as compared to—81.5 mM per kilo, the average normal figure. The gains in chloride and sodium were "equivalent" when calculated on the basis of their concentrations in serum; hence the increase in sodium and chloride content can be explained on the basis of edema formation alone.

The average edema content of the toxic dose heart-lung ventricles was 25.3

grams per cent on the basis of chloride analyses. The potassium content when calculated on an edema corrected fresh tissue basis was significantly reduced, 66.1 mM per kilo as compared to 81.5 mM per kilo, the average normal value.

TABLE 2

Heart-lung ventricles, average values

(NO.) HEARTS OF	WATER CONTENT PER 1000 GRAMS FRESH TISSUE	CONCENTRATION IN mM PER:						MINUTES DURATION OF PREPARATION
		Potassium		Sodium		Chloride		
		1000 grams fresh tissue	100 grams of dry weight	1000 grams fresh tissue	100 grams of dry weight	1000 grams fresh tissue	100 grams of dry weight	
	grams							
(15) Normal dogs	783 (766–799)	81.5 (73.4–87.0)	37.4 (31.6–40.0)	35.3 (32.7–39.1)	16.3 (14.3–18.9)	29.0 (24.8–33.0)	13.4 (11.7–15.6)	
(5) Control Heart-lungs	810 (784–835)	66.7 (60.7–75.1)	35.1 (32.3–37.7)	54.8 (47.5–64.8)	28.8 (23.4–39.3)	46.2 (43.5–51.9)	24.3 (20.1–31.3)	152 (105–182)
(8) Toxic dose Heart-lungs	810 (791–852)	50.8 (44.6–66.0)	25.6 (21.8–30.4)	75.6 (52.6–92.1)	40.3 (25.6–57.0)	52.1 (40.3–65.6)	27.4 (19.7–43.2)	207 (92–330)
	Average serum values							
Normal dogs	937	4.69		141		113		
Control Heart-lungs		3.82		144		119		
Toxic dose Heart-lungs		5.72		143		117		

TABLE 3

Calculated electrolyte and water exchanges of heart-lung ventricles, average values

(NO.) HEARTS OF	CALCULATED EDEMA CONTENT FROM:		POTASSIUM CONTENT ON BASIS OF "EDEMA CORRECTED" FRESH TISSUE	Cl GAIN	Na GAIN	Na "SERUM EQUIVALENT" OF Cl GAIN	Na GAIN IN EXCESS OF "SERUM EQUIVALENT" OF Cl GAIN	K GAIN
	Chloride content	Sodium content						
	grams	*grams*	*mM per 1000 grams of fresh tissue*				*mM*	*mM*
(5) Control Heart-lungs	175	173	80.0	20.9	25.0	25.2	−0.2	−1.5
(8) Toxic dose Heart-lungs	253	370	66.1	29.6	48.7	36.3	12.4	−11.6

The gain in sodium was in excess over the "serum equivalent" of the chloride gain. This excess amounted to 12.4 mM per kilo of fresh tissue, and is presumably due to the entrance of sodium into the muscle cell in exchange for the lost po-

tassium. The loss in potassium was 11.6 mM per kilo of fresh tissue, approximately the chemical equivalent of the sodium gain.

A better concept of the actual cardiac muscle cell exchanges which occurred can be gained by calculating the "extracellular water"[3] content of these heart-lung hearts. This makes possible a calculation of the approximate intracellular space composition as described by Hastings and Eichelberger (14).

In calculating the "extracellular water" of the heart-lung ventricles it was assumed that the extracellular fluid had the same protein content as the serum

TABLE 4

Comparison of the extracellular and intracellular composition of normal dog ventricles, control heart-lung ventricles, and heart-lung ventricles from preparations which received a toxic dose of a digitalis glycoside

	GRAMS H_2O/ KGM. OF FRESH TISSUE	POTASSIUM	SODIUM	CHLORIDE
15 normal ventricles				
		mm. per kgm. of H_2O		
Whole ventricle	783	104	45.2	37.1
Extracellular space: (23.2 grams per cent)	990	4.74	145	127
Intracellular space: (76.8 grams per cent)	721	145	3.77	0.0
5 heart-lung ventricles—no drug				
Whole ventricle	810	81.4	67.6	57.0
Extracellular space: (38.8 grams per cent)	937	4.08	154	127
Intracellular space: (61.2 grams per cent)	729	146		0.0
8 heart-lung ventricles—toxic doses of a digitalis glycoside				
Whole ventricle	810	62.7	93.3	64.3
Extracellular space: (44.6 grams per cent)	937	6.10	153	125
Intracellular space: (55.4 grams per cent)	710	122	29.9	0.0

(11) and therefore Donnan factor corrections could be neglected. The relation then becomes:

$$\text{Per cent extracellular water gram} = \frac{100\ (\text{mM per kilo ventricular chloride})}{(\text{mM per kilo serum chloride})}$$

The average results of these calculations are given in table 4. The "extracellular water" of heart-lung ventricles is significantly increased over normal due to edema formation which is extracellular in position. The differences in the "extracellular water" values for the control and the toxic dose heart-lung ventricles are probably due to the longer duration of the toxic dose preparations (table 2) rather than to an effect of the digitalis glycoside.

[3] "Extracellular water" has been used (15) as a non-committal term for the calculated chloride space, since this calculated value is actually neither the "chloride space" nor "extracellular space" but only a good approximation of these two phases of tissue.

Intracellular water, potassium, sodium and chloride content of control heart-lung ventricles is apparently not significantly different from the normal ventricle. The intracellular composition of the toxic dose ventricles, however, shows definite and significant variations from the controls and normal ventricles. The intracellular potassium content is significantly decreased; 122 mM per kilo of water as compared to 145 mM per kilo of water, the average normal value. The intracellular sodium content is increased. The loss of intracellular potassium is apparently an exchange with an equivalent amount of sodium, since the average increase in intracellular sodium content of 26 mM per kilo of water is approximately chemically equivalent to the corresponding intracellular potassium loss of 23 mM.

Discussion. The bearing of these cardiac electrolyte studies on heart failure and digitalis action are of some interest. The uniformity and magnitude of the cardiac edema which was found to occur in these preparations lends some support to Rühl's contention (16) that cardiac edema is at the basis of heart failure in the heart-lung preparation. However, since digitalis glycosides reverse the processes of failure and yet do not decrease edema, it is equally probable that the edema is an independent concomitant of spontaneous failure.

The finding that untreated failing heart-lung hearts lose little if any intracellular potassium does not agree completely with the results obtained in human heart failure (1, 2). This may result from the fact that failure in the heart-lung heart is much more rapid than that usually seen in clinical heart failure. It should be pointed out that chloride analyses were not carried out in the human heart studies so that calculations of the increase in extracellular space could not be made. Most types of clinical heart failure are associated with increases in the amount of connective tissue and of varying degrees of edema in the heart muscle; until these factors are determined results indicating a decrease in cardiac muscle cell potassium should be accepted with reservations. The fact that nearly all cardiac patients are treated with digitalis may also contribute to the decrease in cardiac potassium concentration which has been reported for such patients.

The electrolyte studies on the heart-lung ventricles receiving toxic doses of digitalis glycosides are in essential agreement with previous results obtained with preparations of other types. The sodium and chloride analyses carried out in this study have added additional information not available in previous studies. It is of interest that the ventricular muscle electrolyte exchanges which were found to result from digitalis action are very similar to the exchanges which occur in striated muscle during activity.

Due to cardiac edema the results concerning the effects of therapeutic doses of digitalis on the potassium content of the heart-lung ventricle are not as conclusive as could be desired. It can be definitely concluded, however, that therapeutic doses of digitalis did not cause an increase in the ventricular potassium content of this preparation. Actually the evidence gives some indication that "therapeutic dose hearts" had a decreased potassium content. This inability to confirm the findings of Boyer and Poindexter (9) on the intact cat and the findings of Hagen (6) on the Langendorff perfused rabbit heart may be due to the dif-

ferences in the preparations used. It may be significant that the average normal potassium content for the cat heart as given by Boyer and Poindexter is 17.0 mM per kilo of fresh tissue lower than the values reported from other laboratories (17, 7, 18).

SUMMARY

Studies of the electrolyte and water content of the heart-lung ventricle are reported.

The ventricles of the untreated (failing) heart-lung heart have: 1, an increased water content; 2, an increase in the wet and dry weight sodium and chloride concentrations; 3, a decrease in the wet and dry weight potassium concentration. Correction for edema and calculation of the "extracellular water" on the basis of chloride analyses indicate that: 1, the intracellular water and electrolyte content of the untreated heart-lung ventricles is not significantly different from normal ventricles; 2, the average edema content is 17.5 grams per cent of the final ventricular weight and is extracellular in position; 3, the increase of sodium and chloride content of these hearts can be accounted for on the basis of the extracellular edema formation.

The ventricles of heart-lung preparations which received therapeutic doses of a digitalis glycoside have: 1, an increased water content which is comparable to the control heart-lung ventricles; 2, an apparent decrease in potassium content which is larger than that found in the control heart-lung and does not appear to be accountable for on the basis of an increased edema content alone.

The ventricles of heart-lung preparations which received toxic doses of a digitalis glycoside show significant changes in their intracellular electrolyte compositions. The intracellular potassium content is decreased apparently in exchange for sodium since there is approximately a chemical equivalent increase in intracellular sodium content. The doses of cardiac glycosides used in these experiments did not significantly affect the water gain or edema formation which occurs in heart-lung ventricles.

The unphysiological factor or factors which are responsible for cardiac edema formation in the heart-lung preparation must exert their chief effect on the capillary membrane, since the electrolyte concentration gradients across the muscle cell membrane and the intracellular water content of untreated heart-lung hearts are apparently normally maintained.

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THE NORMAL PNEUMOCARDIOGRAM

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A simple method for obtaining the pneumocardiogram using an ordinary pneumograph was described in a previous paper (Blair and Wedd, 1939). The principal purpose of that study was the calibration of the record in order to determine the excess of arterial blood which left the chest during the early part of systole, not compensated for by venous return. The observations now reported relate the pneumocardiogram, through the electrocardiogram and heart sounds, to venous and arterial pulses and to the mechanical events of the heart cycle.

EXPERIMENTAL METHODS. The pneumogram of the chest was obtained by placing the pneumograph around the subject's chest at the level of the 5th or 6th ribs. It was connected by rubber tubing to a Frank segment capsule covered with a rubber membrane. A concave mirror of 1 m. radius of curvature placed on the membrane permitted its movements to be recorded photographically by means of a light beam. The segment capsule was placed at the level of the optical system of the string galvanometer which was used to record the electrocardiogram. A small leak through a capillary tube was left in the pneumograph system so that the light beam would return to the camera when the breath was held. When a more accurate wave form was desired the leak was stopped after the light beam returned to the camera. Simultaneity of pneumocardiogram and electrocardiogram was determined by tapping the subject's arm while it was lying across his chest with both recording instruments attached. In this way it was found that the lag in the pneumograph system was less than 0.01 sec. and no correction for simultaneity was considered necessary. Arterial and venous pulses were recorded with the same apparatus, using a receiving tambour instead of the pneumograph. The abdominal pneumogram was recorded by placing the pneumograph around the abdomen at the level of the umbilicus. Neck and thigh pulses were also obtained by use of the pneumograph. For consistency the records from parts of the body other than the chest have been called pneumograms when they were obtained by means of the pneumograph. With normal subjects, placing of the pneumograph at different levels has been found to change only the magnitude and not the direction of the records. In the case of the chest for example, the movements of a light beam reflected from a mirror on the sternum show volume changes similar to those recorded with the pneumograph. The two sides of the chest, however, do not usually move exactly together. Heart sounds were recorded by means of a crystal microphone, amplifier and oscillograph. Pneumograms may be recorded electrically. An example of this type of record is given in figure 8B.

The natural frequency of the pneumograph used was about 12 cycles per second and that of the Frank capsule 2 or 3 times as great. The natural fre-

quency of the chest or abdomen is probably much lower than that of the pneumograph. It is to be expected, therefore, that the apparatus will record accurately volume changes of the part of the body to which it is applied but the body movements will overshoot the forces impressed by the action of the heart or the circulating blood. Consequently for the most part it will be possible to derive from the records accurate information about only the onset of cardiac and circulatory events and not about their course.

EXPERIMENTAL RESULTS. *Heart sounds and chest pneumograms.* In figure 1A is given a record of the heart sounds, the electrocardiogram and the thoracic pneumocardiogram. Small vibrations will be seen in the pneumogram during and following the Q-R-S interval. These are associated with isometric contraction and the beginning of ejection. About 0.14 sec. after the beginning of Q-R-S the pneumogram rises rapidly. This records the rapid collapse, *B*, of the

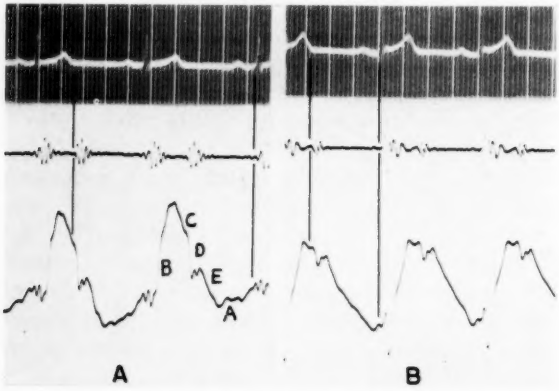

Fig. 1A and 1B. Pneumograms of the chest of two different subjects, with heart sounds and electrocardiograms. An upward movement in the pneumogram indicates a decrease in chest volume. Time marks at 0.04 sec. intervals.

chest due to the outflow of arterial blood. Near the peak of the T wave the collapse reaches its maximum and the chest then expands because the venous return now exceeds the arterial output. The time of the maximum and the form of the wave in this region vary in different subjects and in the same subject in different positions. These points will be discussed later. Corresponding to the beginning of the second sound there is at *C* a small change in slope in the pneumogram which is taken to indicate the closure of the semi-lunar valve. The mechanical unimportance of this event is a tribute to the efficiency of the aortic valve. Following the valve closure is a steep descent of the record, *D*, ending in a plateau with small vibrations. This large movement is considered to be due to relaxation of the ventricles. The reason for ascribing this event to ventricular relaxation is that it just follows the second sound, at which time relaxation is expected. The movement is too late to represent aortic valve closure by a backward movement of the blood in the aorta for that would have to occur before

the second sound and it is too early to be due to ventricular filling. Table 1 shows the relation of the relaxation wave to the beginning of the second sound in a series of subjects. Other reasons for believing this event to be ventricular relaxation will be adduced later. The rapid descent, *E*, following this plateau is due to rapid inflow to the chest of venous blood after the opening of the A-V valves. Following the rapid inflow of venous blood in this particular subject, the thoracic volume reaches its maximum late in diastole and then recedes slightly before auricular contraction occurs at *A*.

It will be seen that the first heart sound begins very soon after isometric contraction begins and that it continues until the large collapse movement of the chest is over. It seems likely, therefore, that in interpreting the low frequency components of the first sound the thoracic wall movements and the factors giving rise to them must be taken into consideration.

The second heart sound lasts through ventricular relaxation and the small vibrations immediately after it. These events probably contribute to the second sound.

TABLE 1

The intervals in seconds between the peak of the T wave and the second heart sound and the beginning and end of ventricular relaxation in a group of normal subjects

2ND SOUND	RELAXATION BEGINS	RELAXATION ENDS	2ND SOUND	RELAXATION BEGINS	RELAXATION ENDS
0.05	0.08	0.10	0.13	0.13	0.16
0.02	0.04	0.09	0.08		0.20
0.10	0.14	0.16	0.07	0.09	0.13
0.10	0.12	0.16	0.10	0.13	0.16
0.10	0.13	0.15	0.09	0.09	0.13

Figure 1B is a similar record from another subject. In this instance, collapse of the chest is preceded by a well-marked expansion or apex thrust. The notch just beyond the summit occurs at the time of aortic valve closure and marks this event more distinctly than in the preceding case. Relaxation of the ventricles gives a smaller excursion and venous return during diastole is slower, as shown by the more gradual expansion of the chest back to the baseline. It should be observed that the movements in the pneumogram may be of two kinds. The general trend of the wave at any time is determined by whether or not the thorax is, on the whole, gaining or losing blood. Mechanical events within the thorax such as rotation of the heart and isometric contraction or relaxation may superimpose vibrations without causing net volume change. In normal subjects, for example, ventricular relaxation appears initially to cause rapid expansion of the chest but on the whole during this event the net volume change is probably only that determined by blood movements.

Venous pulse and chest pneumogram. Figure 2A shows a venous pulse from the neck with the thoracic pneumocardiogram. The waves of the venous pulse are marked *a*, *c*, and *v*, following the terminology of Mackenzie. It will be seen in the pneumogram that a small expansion of the chest begins with the P wave.

This is probably due to auricular contraction. The chest collapses somewhat at the time the neck swells to give the *a* wave, indicating some venous regurgitation. The *c* wave is not well enough marked in this record to relate it to the pneumogram. It will be seen that the downstroke of the pneumogram attributed to ventricular relaxation is followed by the rapid rise of the *v* wave. It is concluded from this that the rise of the *v* wave is due to an effect of the relaxing ventricles on the large veins and auricles. The rapid expansion of the chest, E, due to rapid venous return consequent to the opening of the A-V valves, begins about 0.04 sec. before the downstroke of the *v* wave.

Figure 2B is a venous pulse and a chest pneumogram from another subject. The pneumogram in this case differs in that it has two maxima. It is thought that the expansion of the thorax between these peaks is due to the venous return being larger during this phase of systole than in the subject of figure 2A. The re-

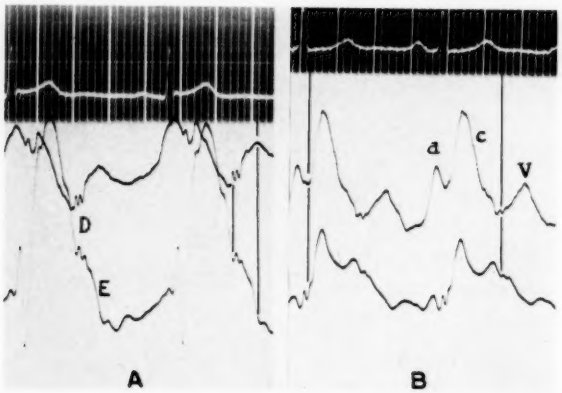

Fig. 2A and 2B. Chest pneumograms from two different subjects—lowest curves in figures, with venous pulses from the neck. An upward movement in the pneumogram indicates collapse of the chest, while an upward movement in the pulse indicates an increase in volume. Time marks at 0.04 sec. intervals. The subject of figure 2A is the same as 1B.

lations of the other events are similar to those in 2A and it can be seen that the *c* wave begins to rise at about the same time the chest begins to collapse.

It will be seen in figure 2A in which the *c* wave is small that the lowest venous pressure occurs about the end of ventricular systole. On the basis of cardiodynamics alone, it would be expected that the venous pressure would be higher at this time than at any other, except possibly at the peak of the *a* wave, because the A-V valves being closed, blood has been accumulating in the auricles and great veins all through systole. The explanation of this anomaly is thought to be given by consideration of the heart as a pump within a closed system. When arterial blood leaves the chest it lowers the pressure there. This lowering of pressure will be compensated by an inward movement of the chest wall, an inward flow of venous blood, or an upward movement of the diaphragm or, most probably, by all of these acting together. That the chest wall does collapse is

shown by the pneumogram. That venous blood is aspirated into the chest is thought to be shown by the anomalously low venous pressure at the end of systole. This point will be elaborated further in the discussion of the abdominal pneumogram.

Venous pulse and the abdominal pneumogram. In figure 3A are shown the venous pulse and abdominal pneumogram. An upward movement in the pneumogram corresponds to a decrease in the size of the abdomen. It will be seen that the general form of the pneumogram is similar to that of the chest. Usually it begins to rise later because the arterial pulse in the abdomen tends to make it expand early in ejection and the collapse is delayed until the venous movement to the chest exceeds the arterial inflow to the abdomen. In this particular case, the arterial pulse causes little or no effect. It is not possible to determine from these records alone how much of the collapse of the abdomen is

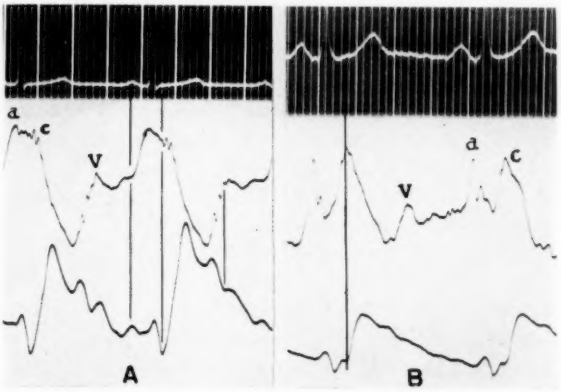

Fig. 3A and 3B. Abdominal pneumograms—lowest curves in records, with venous pulses from the neck. An upward movement in the pneumogram indicates a decrease in volume of the abdomen. Time marks at 0.04 sec. intervals. The subject of figure 3B is the same as 2B.

due to the upward movement of the diaphragm and how much to the movement of venous blood. In either case, however, it is indicated clearly that arterial blood leaving the chest exerts a strong aspirating effect on the abdomen. This same effect must of course be exerted on the veins of the neck. This accounts for low venous pressure at the end of systole. During systole, blood is aspirated from the neck and abdominal veins which process distends the auricles and the veins of the thorax and drains the veins of the abdomen and neck. Following systole, the relaxation of the heart and the decrease of the aspirating action diminish or even reverse the venous inflow so that venous blood accumulates in the neck and the pressure rises to give the upward swing of the *v* wave. This addition to the older views on the venous pulse (Lewis, 1925; Mackenzie, 1925) appears to make its explanation much more satisfactory.

Considering the abdominal pneumogram in more detail, it will be seen that

corresponding to the *a* wave of the neck pulse, there is only a very small downward excursion or expansion of the abdomen. This wave cannot be compared with the venous *a* wave because the recorder is different, but it may be observed that it is small compared with the later abdominal collapse.

In figure 3B, from another subject, a relatively small auricular wave will be seen also. In both 3A and 3B isometric contraction of the ventricles causes rapid expansion of the abdomen which is probably due to the transmission of the apex thrust through the diaphragm. In 3B, a second small expansion is seen just following the first. This is commonly found. It is due to the arterial pulse in the abdomen and it is usually quite small relative to the subsequent collapse.

In figure 3A it will be noted that the initial collapse is followed by a much greater expansion than in 3B. It is not possible to give precise reasons for the variation in wave form because so many factors are involved—arterial flow to and from the abdomen, venous flow to and from the abdomen along with probable movements of the diaphragm. It can be definitely stated, however, that since they both collapse during systole, the chest and abdomen together lose blood to the periphery and regain it during diastole.

Following the second maximum in both 3A and 3B is a phase of rapid expansion occurring at about the same time as the rise of the venous *v* wave. It is concluded that this is due likewise to the relaxation of the ventricles. It occurs later in the abdomen than in the chest by an amount which is similar to that required for transmission to the neck from the chest.

Unlike the neck veins, the abdomen continues to expand after the vibration due to ventricular relaxation rather than to collapse simultaneously with the *v* wave. This may not mean that no additional venous blood moves from the abdomen to the chest during this phase but rather that the diaphragm moves downward to compensate for the movement of the blood.

The neck pneumogram. Since the chest and abdomen collapse during systole, the extremities must, of course, expand. In figures 4A and 4B are given two examples of neck pneumograms along with those of the chest. The upward movement in the neck record is due to increase of volume, not decrease as in the chest records. It will be seen in 4A that the neck begins to expand before the chest begins rapidly to collapse. This initial phase of neck expansion is probably due in some way to the apex thrust. The later rapid phase begins with the chest collapse. The rapid descent denoting ventricular relaxation occurs almost simultaneously in the neck and chest records. The deep notch in the neck record just following ventricular relaxation is taken to be the beginning of the venous *v* wave. This can be seen better in figure 4B.

The neck pulse of 4A is largely arterial in form but in 4B, from another subject, the venous pulse waves are more plainly marked. The conditions necessary to record venous waves have not been investigated carefully but it is known that they develop in certain subjects after the breath has been held for some time. In this subject, the apex thrust being ineffective, chest collapse occurs almost simultaneously with the swelling of the neck. The relaxation wave reaches the neck and the chest wall at about the same time. The time required for transmission

along the arteries and veins will depend on the blood pressures and the condition of the vessels, while the time required for the thorax to respond will depend on similar factors involving the elasticity of the lungs, et cetera, consequently, it is not to be expected that there will be uniformity of relationship between the cardiac events as recorded from the neck and from the chest.

Carotid pulse and thoracic pneumogram. In figure 5A is shown a carotid pulse almost superimposed on the chest pneumogram which enables their relations clearly to be perceived. In this case there is almost exact simultaneity between the collapse of the chest and the swelling of the carotid, and between the ventricular collapse wave in the chest and the notch in the pulse. These waves have the similarity in form to be expected in a subject in whom the venous return has no sudden variations during the cycle for in such a case the form of the chest movement will be determined largely by the variable arterial flow.

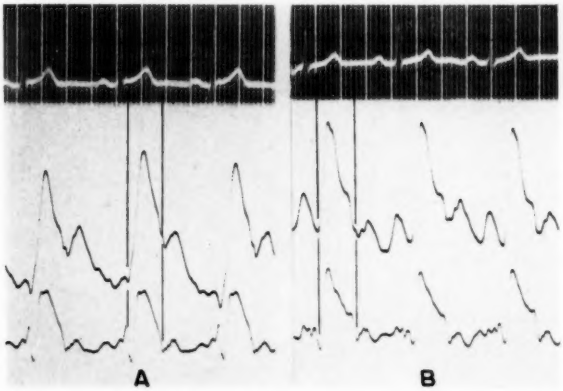

Fig. 4A and 4B. Chest pneumograms—lowest curves, with pneumograms of the neck on two different subjects. An upward movement in the neck record corresponds to an increase in volume; in the chest record it corresponds to a decrease. Time marks at 0.04 sec. intervals. The subject of 4A is the same as 1B and 2A. The subject of 4B is the same as 1A.

In figure 5B, from another subject, it is seen that the collapse of the chest lags behind the swelling of the carotid about 0.02 sec. The early phase of the carotid expansion is probably due to the apex thrust as in figure 4A. The later rapid rise coincides with chest collapse. The end of the collapse wave in the carotid is shown somewhat earlier. This record is from an older and stouter subject than that of 5A. Transmission from the heart to the chest wall may be relatively slower, or the chest wall may have greater inertia. The form of the chest wave differs considerably from that of the pulse. This is probably due to a large inflow of venous blood just following the initial collapse of the chest.

Attribution of the notch in the pneumogram to relaxation of the heart necessitates the same interpretation, in part at least, of the incisura of the arterial pulse. Without discussing in detail all the possible mechanical events associated with semi-lunar valve closure, it appears desirable here to stress the part played by

ventricular relaxation. At the moment that the semi-lunar valves have just closed, the pressures on either side are approximately equal. From this moment on ventricular pressure drops rapidly toward zero, taking away the support on the ventricular face of the valve. Consequently, the relatively high aortic pressure will cause the valve to bulge rapidly and the system, as a whole, to oscillate. The notch in the aortic pulse is probably the primary oscillation of this movement. With the thorax open, ventricular relaxation subsequent to the bulging of the valve may not have much additional effect on the arterial system. With the chest closed, however, it is clear from the evidence above that the effects of mechanical events within the chest are transmitted rapidly throughout the whole trunk. Consequently, the arteries within the trunk are subjected to all these effects. Considering that the mass of the ventricles is several times as great as the total stroke volume of the heart, the results of its movement are

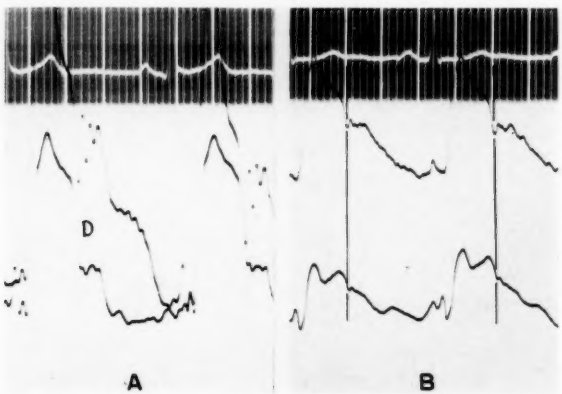

Fig. 5A and 5B. Chest pneumograms of two different subjects—lowest curves, with carotid pulses. Time marks at 0.04 sec. intervals. The subject of figure 5B is the same as 2B and 3B.

likely to be important. For these reasons, ventricular relaxation is thought to be the principal factor in incisura production both through its effect on the aorta by permitting the valve to bulge and through the transmission of its later stages by the closed thoracic system.

Relation of isometric contraction and chest collapse to Q-R-S. In figure 6 are plotted the beginning of isometric contraction, the end of the S wave and the beginning of chest collapse for a group of normal subjects, all measured from the beginning of the Q-R-S complex. The beginning of contraction was taken in each case to correspond to the first vibration in the chest pneumogram following the beginning of electrical systole; for example, the one beginning at the right hand timing line in figure 1A. Any lag in the larger waves of the chest pneumogram does not necessarily operate for these small vibrations because they may be transmitted as sound waves through the lungs. There is a possibility that they, in some cases at least, may be due to auricular relaxation, but usually they are

much sharper than anything observable at the time of auricular contraction. Also, in most cases, the beginning of the first sound comes just after these movements while there is very little sound recorded from auricular contraction. Assuming that these vibrations indicate the beginning of the isometric phase, it is found that this phase begins, as will be seen in figure 6, in many subjects at the same time as the beginning of electrical systole. In those cases in which the beginning is apparently late it is possible that the first mechanical movements are not well transmitted. It is concluded, consequently, that mechanical and electrical systole begin very close together.

It will be observed at the top of the figure that the time between the beginning of electrical systole and the collapse of the chest varies in normal subjects from 0.1 to 0.15 sec., the average being 0.126 sec. This time includes isometric contraction along with the time required for arterial blood to begin to leave the chest

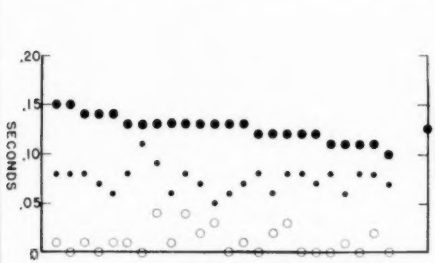

Fig. 6

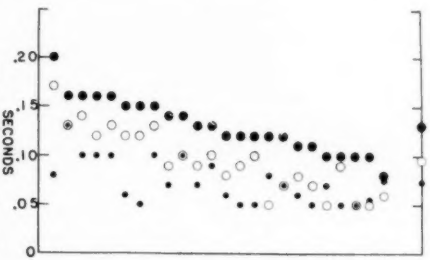

Fig. 7

Fig. 6. The beginning of isometric contraction—large circles, end of the Q-R-S complex—small dots, and the beginning of chest collapse—large dots; all measured from the beginning of electrical systole. The three data of each column are from a different subject. The average collapse time is given by the separated dot at the right.

Fig. 7. The end of the T wave—small dots, beginning of isometric relaxation—large circles, end of isometric relaxation—large dots; all measured from the peak of the T wave. The three data from each column are from a different subject. The averages are given by the separated symbols at the right.

and any lag in the chest wall. It has not been possible to ascribe any significance to the differences in normal collapse times except that it is known that the collapse is very early in the fast beating hearts of hyperthyroidism. The duration of Q-R-S does not seem to be a factor in determining collapse time as will be seen from the middle of the diagram.

Relation of relaxation to the T wave. In figure 7 the intervals from the peak of the T wave to the end of the T wave and to the beginning and end of relaxation are plotted. It will be observed that there is a wide variation in the interval between the peak of T and relaxation. On the other hand, the interval from the end of T to relaxation is much more constant. In two-thirds of the cases relaxation begins after the end of the T wave, while in one-sixth they begin together and in the remainder relaxation begins before the end of the T wave. These differences may be due in part to the fact that neither the end of the T wave nor the beginning of relaxation is easily determined in some subjects. On the aver-

age, the T wave ends 0.074 sec. beyond its peak while relaxation begins at 0.095 sec. and ends at 0.13 sec.

Auricular contraction and the chest pneumogram. Usually the effect of auricular contraction on the chest wall is small but it can be considerable. In figure 8A is shown a chest pneumogram and the venous pulse from the neck. The collapse of the chest occurring with the *a* wave of the venous pulse is almost as large as that due to the ejection of arterial blood. This illustrates in another way that blood migration, in this case venous regurgitation, is the factor which gives rise to the principal volume changes of the chest rather than any direct mechanical action of the ventricular pump itself. The second heart sound occurs in this subject, according to separate determination, just before the first small rebound at the bottom of the first long descent of the pneumogram below the T wave of the

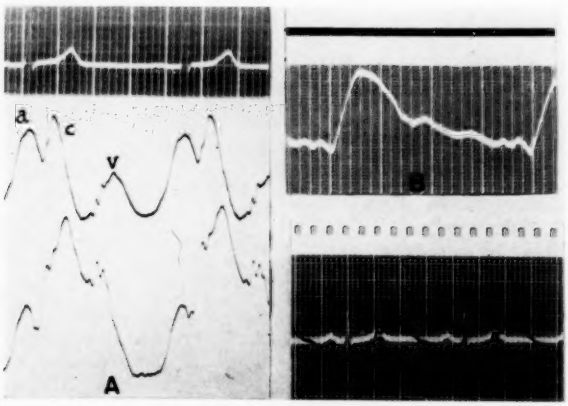

Fig. 8A. Venous pulse—upper record, pneumogram of chest showing large auricular effect—lower record.

Fig. 8B. Pneumogram of chest from subject in sitting position, recorded electrically.

Fig. 8C. Pneumogram of thigh. Upward movement denotes increased volume.

electrocardiogram. The ensuing ventricular relaxation gives rise to further collapse of the chest indicating that temporarily during this phase venous return is reduced to a value below arterial output. This is a clear demonstration that the relaxing heart impedes the venous return in the manner which produces the rise of the *v* wave.

Chest pneumogram recorded electrically. In figure 8B is a chest pneumogram taken in the sitting position from the subject of figure 2B by means of the Sanborn heart sound microphone driving the electrocardiograph channel of the Sanborn Stetho-Cardiette. It will be seen that the pneumogram is more regular in outline than in figure 2B. The difference in records from the sitting and the supine positions are much more pronounced in some subjects than in others. They are probably determined by differences in venous blood movements.

The leg pneumogram. In figure 8C is a pneumogram from the thigh. The

thigh increases in volume following systole, beginning usually about 0.2 sec. after the beginning of Q-R-S or about 0.07 sec. after the chest starts to collapse. The form of the thigh pneumogram differs considerably in different subjects. Frequently there are several relatively large oscillations on the main wave. The amplitude of the pulse is much smaller than that obtained from the neck with the same apparatus. The neck probably acts as an elastic arterial reservoir to a greater extent than any region of the limb because it supplies the relatively inextensible head.

The effect of arterial ejection on venous return. It will be seen that if the chest were rigid and full of fluid the ejection of blood by the heart would have to be accompanied by equal venous return or a vacuum would be created which would make ejection possible only if the force of the pump exceeded atmospheric pressure. Ejection of 60 cc. of blood from an air-filled rigid chest of 3000 cc. capacity, unaccompanied by venous return would create a drop in pressure of one-fiftieth atmosphere or about 15 mm. Hg. This is the maximum possible aspirating force promoting venous return. This maximum will not be reached because the chest is not rigid and because venous blood does return. The actual aspirating pressure is sufficient, as was previously shown (Blair and Wedd, l.c.) to cause about one-half the venous return to occur during ventricular ejection, i.e., in about one-third of the heart cycle. It is indicated by this fact that aspiration of venous blood is an important factor in circulation.

SUMMARY

Pneumograms of the chest, the abdomen, the neck and the thigh taken during suspended breathing are related to the electrocardiogram and to venous and arterial pulses. Ejection during systole of arterial blood from the chest creates there a fall in pressure which causes collapse of the chest wall, a probable rise of the diaphragm and aspiration of venous blood from the neck and abdomen. The effect on the abdomen is such that it also collapses. The volumes of the neck and thigh increase to compensate for the decreased volume of the trunk. During diastole, the chest and abdomen expand because venous return exceeds arterial outflow. When decrease of the volume of the chest is recorded in the same direction as increase of pressure in the artery, the pneumocardiogram of the chest is similar in form to a record of the carotid pulse. The pneumogram usually indicates the beginning of isometric contraction of the ventricles, the beginning of ejection from the chest, the beginning and end of isometric relaxation, auricular filling and sometimes auricular contraction. It yields, in general, more information than the pulse while it is much easier to record. It is concluded that the low pressure of the neck veins during ventricular systole is due to the aspirating action of arterial ejection. Aspiration of venous blood is probably an important factor in promoting venous return. The rise of the venous *v* wave is thought to be due to ventricular relaxation. The part played by relaxation of the ventricles in producing characteristic waves in the pneumograms of the chest and abdomen and the notch in the arterial pulse is stressed. The relation of the Q wave of the electrocardiogram to the beginning of ventricular contraction and

of the T wave and the second sound to ventricular relaxation is determined for a group of normal subjects. Electrical and mechanical systole are seen usually to begin very close together.

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TITRATIONS OF HUMAN SEMINAL FLUID WITH ACIDS AND ALKALIS AND THEIR EFFECTS ON THE SURVIVAL OF SPERM MOTILITY

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Many problems dealing with fertility, artificial insemination and contraception involve a knowledge of the environmental conditions which affect the survival of human spermatozoa. It has been known for some time that among these conditions, the pH of the surrounding medium is an important factor which requires further study. Muchat (1) indicated that the optimum pH for motility of human spermatozoa lies between pH 8.5 and 9.5 and that motility stops when the pH is less than 6 or greater than 10. Voge (2) reported spermicidal tests with human semen and lactic acid. He noted that a pH of 4.9 to 6.4 arrests sperm motility and that below pH 3.5 revival by neutralization is not possible. All his pH measurements were carried out colorimetrically which may involve some ambiguities. Baker (3a) reports that all cavy spermatozoa are immobilized in thirty minutes by solutions of sulfuric, acetic, tartaric and citric acid when the pH of the medium is below 5. Since the specimens in his tests differ in both the medium and species from human semen, direct comparisons with human spermatozoa cannot be made. Shedlovsky (4, 5) summarizes the meager available information on the spermicidal action of acids as dependent on the pH and buffer capacity of the fluids involved.

The maintenance of a favorable pH or the attainment of one which is lethal to spermatozoa will depend on the buffer capacity of seminal fluid. The only quantitative data which we found in this connection are those of Baker (3b, 3c), Baker, Ranson and Tynen (6) and Tynen (7) who established several points on the titration curve, with hydrochloric acid but only down to pH 6.0, and Schersten (8) who made two determinations to a pH of 4.9. Our report covers a much wider range of pH since we were also interested in spermicidal effects.

It has been our object to make a more precise and complete study of the buffer capacity of semen and the effect of changing the pH of the medium on the survival of the motility of spermatozoa.

EXPERIMENTAL. The semen specimens were obtained in glass vials from 12 donors. Precautions were taken to avoid using a given donor more than once a week. Spermicidal tests were begun on individual specimens within two hours from the time they were obtained and carried on for several hours. The age of the specimen affects the spermicidal tests sufficiently to require that a series of such tests should be started within an interval of about one hour in order to have the results of a particular series on a comparable basis (9a).

[1] This work was made possible by a grant from the National Committee on Maternal Health.

A series of solutions of hydrochloric, lactic, citric, tartaric, maleic, phosphoric, acetic and monochloracetic acid and sodium and potassium hydroxide and ammonia of known concentrations was prepared by the dilution of standardized stock solutions. In order to distinguish between the spermicidal effects due to the pH and those due to changes in salt concentration, all the stock solutions and dilutions made from them contained 0.9 gram per 100 cc. (0.154 M) NaCl except for KOH which contained 1.15 grams per 100 cc. (0.154 M) KCl.

Equal volumes of either 0.10 or 0.15 cc. of semen and acid or alkali solution were measured out from 0.5 or 1.0 cc. tuberculin syringes and mixed thoroughly by drawing the mixture into the syringe and expelling it into a 3 cc. vial ten times. The use of such small amounts permitted many tests to be made with each specimen. A small drop of the mixed fluids was placed upon a slide, a cover slip placed on it and the preparation examined by means of dark field illumination. Observations were made with both low and high power magnification until every spermatozoön had become immotile, so that both translational as well as vibrational motion was absent. The number of minutes taken for this to occur from the time mixing was started was defined as the spermicidal time.

As a control test, equal volumes of semen and 0.14 molar (2.8 per cent) aqueous solution of potassium acid phthalate (initial pH 4.0) were mixed (9b). The pH of such mixtures for various specimens ranged from 5.0 to 5.4. A specimen was not suitable for comparative spermicidal tests when the spermicidal time for such a mixture was less than twenty minutes. These tests were performed at room temperatures (20–26°C.).

The pH measurements were made with a shielded glass electrode assembly of the type described by MacInnes and Longsworth (10) using a Compton Electrometer and a L. and N. Student Type Potentiometer. The condenser shaped electrode had a capacity of 0.25 cc., and it was possible to make measurements with less than this volume. The introduction of the mixture into the electrode was facilitated by having the top of the latter ground to fit the standard tip of the syringes. It was possible to make these measurements with a precision of ± 0.01 pH unit.

As a standard of pH we have adopted 0.05 molar potassium acid phthalate solution, which has a pH of 4.00 at 25°C. To insure the correct performance of the electrode, it was always checked with another known phosphate buffer (pH 7.73) and 0.1 N HCl (pH 1.08) before and during the experiments. After a considerable number of determinations, there was a tendency for the electrode to give values too low by 0.1 to 0.2 pH unit, but this error due perhaps to protein films was obviated by washing the electrode immediately after each determination with a little dilute HCl solution (0.1 N), and following with a considerable amount of water. If the latter is forced by a 20 cc. syringe through the electrode at high velocity, it tends to remove films.

The carbon dioxide content of the semen was determined by the Van Slyke volumetric method (11).

Results. The titration curves with HCl and NaOH solutions are given in fig-

ures 1 and 2 respectively. In these curves the pH of the mixtures of acid or alkali with semen are shown as a function of the milliequivalents of acid or alkali added per cubic centimeter of a mixture of equal volumes of semen and acid or alkali. The upper curves (fig. 1) lying fairly close together, indicate what may be expected for the normal run of semen specimens mixed with acid, an occasional curve lying at some distance below the others shows the occurence of a relatively poorly buffered specimen. Eleven additional titrations of semen with HCl were made besides those given in figure 1. In these determinations mixtures of equal volumes of semen and 0.50 N HCl gave a pH range from 0.97 to 1.24 with an average value of 1.1.

The titration curves for the other acids which are not shown gave higher pH values than for the corresponding points on the HCl curves, and a specimen which was poorly buffered against one acid was found to act similarly with the other acids.

Figure 3 summarizes the data for the pH of equal volumes of semen and solutions against the spermicidal time for all the acids tested. Region A covers the range of the results for monochloracetic and acetic acid and region B covers the range of the results for hydrochloric, lactic, tartaric, citric, phosphoric and maleic acid. In each group all the experimental points are entirely in the region indicated, although on the average the spread of values is only about half as great as that shown.

Figure 4 shows typical curves of pH against spermicidal time for tartaric, phosphoric, maleic, citric and monochloracetic acid, for a single semen specimen. In figure 5, for two other semen specimens marked 1 and 2, the difference is demonstrated between monochloracetic and acetic acid against citric and tartaric acid which in general have effects similar to hydrochloric and phosphoric acid.

In figures 3 to 5 it may be noted that spermicidal times above 120 minutes are not indicated. When the time for complete immobilization of spermatozoa is much greater than this, the values cannot be quantitiatively compared with shorter periods of time.

In order to give an example demonstrating quantitatively the differences between acidic solutions of different buffer capacities, we compared solutions of mixtures of lactic acid and sodium lactate and tartaric acid and sodium tartrate with solutions of the corresponding acids. For the buffer solutions, the total concentration of anion constituent was 0.5 normal in each case. No sodium chloride was used in these experiments. To indicate how much less such buffer solutions increase in pH than solutions of the acids alone when mixed with semen, the change for one solution will be given. When a tartaric acid solution with a pH 2.5 is mixed with an equal volume of semen, the pH will be 6.7, whereas for the tartrate buffer it will be 3.5. In each case the spermicidal time is determined by the pH of the mixture so that for pH 6.7 it would be more than 300 minutes and for pH 3.5 about 1 minute for the same specimen of semen.

The characteristic spermicidal effects of alkalis are shown in figure 6 for a particular semen specimen. NaOH and KOH have essentially the same effects, but ammonia solutions show a specific effect apart from the pH.

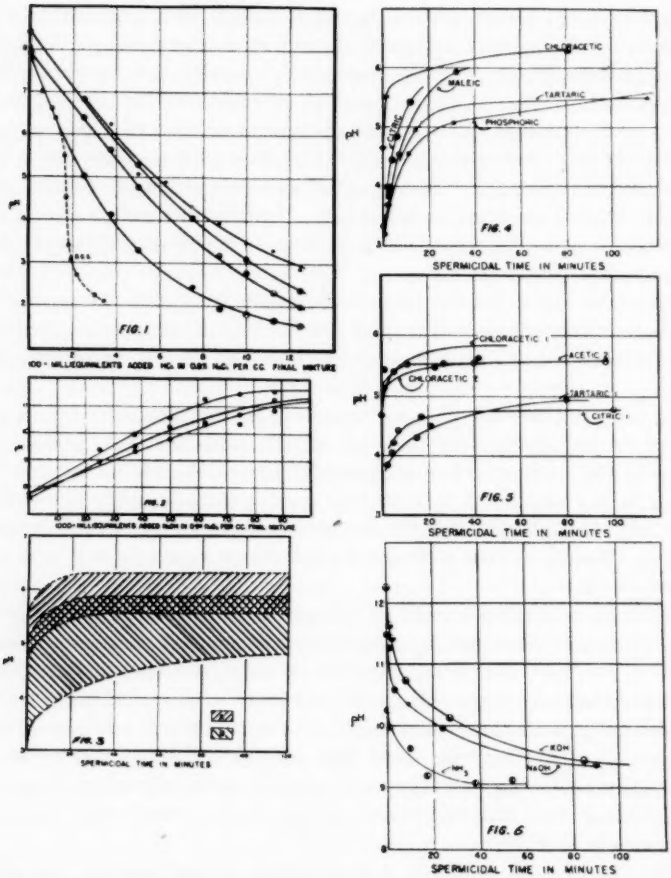

Fig. 1. Titration of semen with HCl solution. pH against milliequivalents added acid per cubic centimeter of final mixture of equal volumes of semen and acid solution. Each curve represents a different semen specimen. Curve marked *B.G.S.* represents titration for Baker's Buffered Glucose Solution (3b).

Fig. 2. Titration of semen with NaOH. pH against milliequivalents added base per cubic centimeter of final mixture of equal volumes of semen and alkali. Each curve represents a different semen specimen.

Fig. 3. Summary of data for acids: pH of mixture of semen and acid solutions against spermicidal time. Region (A) monochloracetic and acetic acid, region (B) hydrochloric, lactic, tartaric, citric, phosphoric and maleic acid. Points for all tests made always fall in the region indicated.

Fig. 4. pH of mixture of semen and acid solutions against spermicidal time for phosphoric, tartaric, citric, maleic and chloracetic acid. All curves for the same semen specimen.

Fig. 5. pH of mixture of semen and acid solutions against spermicidal time for citric, tartaric, acetic and monochloracetic acid. *1* indicates one semen specimen. *2* indicates another semen specimen.

Fig. 6. pH of mixture of semen and alkali solutions against spermicidal time for NaOH, KOH and NH_3. All curves for the same semen specimen.

It has been noted that semen on standing, first becomes more alkaline and our data indicate that this change is most likely due to loss of carbon dioxide. Our measurements of the pH of semen gave a range from 7.6 to 8.4 for various specimens, but in most cases the values were less than 8.0. The result of the experiment given in table 1 shows how the carbon dioxide content affects the pH of the semen. Ageing has been shown by others first to make semen more alkaline and then more acid, the latter effect has been attributed to the formation of more lactic and other acids. The formation of acid was avoided in our experiments (table 1) by the addition of phenyl mercuric nitrate at the start.

DISCUSSION. The pH of seminal fluid as reported by a number of investigators covers a range from pH 7.0 to 8.9 and unless precautions are taken to prevent loss of carbon dioxide the pH increases. We have shown that it is most likely that the higher values were for semen that had lost carbon dioxide on ageing.

TABLE 1

Change in CO_2 content and corresponding pH of semen

(a) Specimen kept in jar with cover on loosely. Phenyl mercuric nitrate added at start of experiment. All sperms immotile.

AGE IN MINUTES FROM TIME COLLECTED	MILLIMOLES CO_2 PER LITER	PER CENT CHANGE IN CO_2 FROM INITIAL VALUE	pH	CHANGE IN pH
8	21.3		7.73	
243	11.1	−48	7.78	+0.05

(b) Composite of two semen specimens with phenyl mercuric nitrate added at time of collection. Temperature 22°C.

TREATMENT	MILLIMOLES CO_2 PER LITER	PER CENT CHANGE IN CO_2 FROM INITIAL VALUE	pH	CHANGE IN pH
Mixture 2 hours old. Kept in covered container	24.8		7.57	
Mixture kept 6 hours over concentrated KOH solution	6.7	−73	7.89	+0.32
Mixture kept 6 hours under 1 atmosphere pressure of CO_2	81.9	+230	6.43	−1.14

In our opinion, the buffer capacity of the semen is far more important than its pH and this value gives no clue to the buffer capacity as can be seen from figure 1. The reports of Baker, Ranson and Tynen (6) give the amounts of 0.01 N HCl necessary to bring the pH of 1 cc. of semen to 7.1, 6.7, and 6.0. Their range of values of the amount of acid added agree well with the corresponding points on our curves (fig. 1) but do not cover an adequate range of pH to indicate its buffer capacity to larger quantities of acid. Schersten (8) reports two titrations of semen with HCl solution using the quinhydrone electrode. He took 1 cc. of a human semen specimen and added 0.1 cc. increments of 0.1 N HCl up to 1.0 cc. and determined the pH. For the last point of 0.10 milliequivalents of HCl per cc. of semen, Schersten obtains a pH of 4.85 which corresponds to our pH values for this point which ranged from 4.8 to 5.4 for different specimens (fig. 1).

It may be of interest to examine the buffer capacity of two artificial fluids which have been used as diluents for semen or suspending media for spermatozoa. One solution of this kind is that of Baker (3b) known as B.G.S. (buffered glucose saline) which has the following composition: KH_2PO_4 0.1 gram, $Na_2HPO_4 \cdot 2H_2O$ 3.0 grams (equivalent to 6.0 grams $Na_2HPO_4 \cdot 12\ H_2O$) NaCl 2.0 grams, glucose 30.0 grams and water to make 1000 cc. The pH of this solution is 8.1 and our titration curve with hydrochloric acid (fig. 1) resembles that of the lowest curve for semen over a small range to pH 6.0, but at lower pH values, it is poorly buffered in comparison even with the least buffered semen specimen tested. Another solution proposed by Baker (3b) is a mixture of 1.4 cc. of 6 per cent $Na_2HPO_4 \cdot 12\ H_2O$ with 1.6 cc. of egg white. This solution compares very favorably with semen as an alkaline buffer but the use of various samples of egg white show considerable variations.

From an examination of the data as well as from spermicidal tests with other substances, it becomes apparent that quantitative comparisons of spermicidal tests can be made only for a particular semen specimen. The most important reasons for this are variations in both the seminal fluid as well as the spermatozoa. For example, the titrations in figure 1 give a range of values for different specimens although each specimen gives a reasonably smooth curve.

For hydrochloric, lactic, citric, tartaric, maleic and phosphoric acid, the spermicidal properties are primarily the same function of the pH of the medium and no significant specific effects are indicated.

Climenko (12) suggests that maleic acid has spermicidal properties which "seem to depend on the fact that it inhibits the oxygen consumption of the cell." His tests were made in buffered glucose saline solution with cavy spermatozoa, but apparently the acidic properties of the maleic acid were not considered a significant factor which determines its spermicidal properties. Our results show that maleic acid has spermicidal properties with human spermatozoa which are comparable to those of citric and tartaric acid and that no marked specific effect is indicated.

Osterhout (13) points out that plant cell membranes are particularly permeable to undissociated molecules. If this argument is applicable to sperm cell membranes, it may lead to a partial explanation of the specific effect of acetic acid and ammonia when compared with stronger acids and alkalis respectively. Monochloracetic acid has a larger dissociation constant than lactic acid, but shows specific spermicidal properties when compared with it. In this case, the specific effect may be related to the inhibition of the glycolysis of spermatozoa comparable to that shown for iodoacetic acid.

SUMMARY

Methods are described for carrying out titrations of semen with acids and alkalis using a glass electrode assembly suitable for volumes as low as 0.15 cc. with a precision of ± 0.01 pH and for the determination of the relative spermicidal properties of such mixtures.

Some comments and measurements are made on the relative buffer capacity of solutions proposed as diluting media for semen.

Changes in the pH of semen as a function of the CO_2 content are shown by keeping the semen over concentrated KOH solution or under one atmosphere pressure of CO_2. The pH varies from 7.9 for 6.7 millimoles CO_2 per liter to 6.4 for 81.9 millimoles CO_2 per liter.

Titration curves and the survival time of the motility of spermatozoa for semen with hydrochloric, lactic, citric, tartaric, maleic, phosphoric, acetic and monochloracetic acid, sodium and potassium hydroxide and ammonia solutions are reported. For the first six acids, each dissolved in solutions containing 0.9 gram NaCl per 100 cc. and mixed with equal volumes of semen, the time for complete immobilization of the spermatozoa was less than half a minute in every case tested if the pH of the mixture was less than 3.0. Acetic and monochloracetic acid show specific spermicidal effects. For these acids, the time for the complete immobilization of spermatozoa is always less than half a minute if the pH of the mixture is less than 4.7.

As an example of the above effects, the marked superiority of the spermicidal properties of well buffered tartrate and lactate solutions over solutions of the corresponding acids alone is shown on a quantitative basis.

Sodium and potassium hydroxides give a time for the complete immobilization of spermatozoa of less than half a minute when the pH of the mixture is greater than 12.2, whereas in the case of ammonia specific effects are indicated so that the same spermicidal properties are obtained when the pH is 10.3 or higher.

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